DOKUZ EYLÜL UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

ECOTOXICITY of
ATMOSPHERIC DEPOSITION

by
Sibel ÇUKURLUOĞLU ÇİZMECİOĞLU

April, 2006
İZMİR

ECOTOXICITY of ATMOSPHERIC DEPOSITION

A Thesis Submitted to the
Graduate School of Natural and Applied Sciences of Dokuz Eylül University
In Partial Fulfillment of the Requirements for
the Degree of Doctor of Philosophy in Environmental Engineering,
Environmental Sciences Program

by
Sibel ÇUKURLUOĞLU ÇİZMECİOĞLU

April, 2006
İZMİR

Ph.D. THESIS EXAMINATION RESULT FORM

We have read the thesis entitled "ECOTOXICITY of ATMOSPHERIC DEPOSITION" completed by Sibel ÇUKURLUOĞLU ÇİZMECİOĞLU under supervision of Prof.Dr. Aysen MÜEZZİNOĞLU and we certify that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Doctor of Philosophy.

Prof.Dr. Aysen MÜEZZİNOĞLU

Supervisor

Prof.Dr. Emür HENDEN	Assoc.Prof.Dr. Mustafa ODABAŞI
Thesis Committee Member	Thesis Committee Member
Prof.Dr. Gürdal TUNCEL	Assoc.Prof.Dr. Abdurrahman BAYRAM
Examining Committee Member	Examining Committee Member

Prof.Dr. Cahit HELVACI
Director
Graduate School of Natural and Applied Sciences

ACKNOWLEDGMENTS

I would like to express my gratitude to my supervisor Prof.Dr. Aysen MÜEZZİNOĞLU for her invaluable advice, guidance and encouragement. Completion of this work would not have been possible without her help. I would like to thank Prof.Dr. Emür HENDEN and Assoc.Prof.Dr. Mustafa ODABAŞI for their guidance, support and encouragement. I am also grateful to Assoc.Prof.Dr. Abdurrahman BAYRAM for his comments and support.

I would like to thank Dokuz Eylül University and The Scientific & Technological Research Council of Turkey (TÜBİTAK) for their support of my Ph.D. studies which includes partial financial backup.

I would like to thank the Dokuz Eylül University Air Pollution Laboratory staff and especially Dr. Remzi SEYFİOĞLU for their valuable help. In addition, I am also grateful to Prof.Dr. Latif ELÇİ, Asst.Prof.Dr. Ümit DİVRİKLİ and Analytical Chemistry Laboratory staff of the Pamukkale University for their valuable analytical help.

I am grateful to Civ.Eng. Dr. Ülker GÜNER BACANLI for her friendship, support, encouragement as well as help in statistical evaluations.

I would like to thank my husband Uğur ÇİZMECİOĞLU for help. I am grateful to my father Nurettin ÇUKURLUOĞLU and my mother Sevgi ÇUKURLUOĞLU for their support and valuable help. My parents are always there for me and are one of the most important factors that shaped me into who I am today. Finally, I would like to thank my daughter Yağmur ÇİZMECİOĞLU for her love, emotional support and patience during my research.

Sibel ÇUKURLUOĞLU ÇİZMECİOĞLU

ECOTOXICITY of ATMOSPHERIC DEPOSITION

ABSTRACT

Dry and wet deposition samples were collected between October 2003 and June 2004 in Buca, Izmir. Dry and wet depositions of selected heavy metals (Cr, Cd, Pb, Cu, Zn and Ni) were measured using a water surface sampler. Dry deposition samples were collected over 24 hours and wet deposition samples were taken over the rainy period.

Dry and wet deposition samples were filtered and both filters and filtrates were analyzed for selected heavy metals using a Perkin-Elmer Model 700 atomic absorption spectrophotometer equipped with a graphite furnace except for zinc which was analyzed by using a flame technique.

The volume weighted average total heavy metal concentrations of Cr, Cd, Pb, Cu, Zn and Ni were found as 17.1±8.5, 3.1±1.6, 6.6±4.1, 19.5±24.8, 184.2±224.0, and 6.7±2.6 in µg L^{-1}, respectively for wet deposition samples. These concentrations were generally higher in Izmir than the values previously measured at different sites around the world.

The average total dry deposition fluxes of Cr, Cd, Pb, Cu, Zn, and Ni were 96.4±69.2, 41.3±20.0, 90.3±40.8, 81.3±48.6, 2127.2±651.4, and 139.6±62.0 µg m^{-2} day^{-1}, respectively. The average total wet deposition fluxes of Cr, Cd, Pb, Cu, Zn, and Ni were 271.2±148.1, 54.3±46.1, 111.5±77.9, 362.5±670.5, 2387.7±2807.2, and 107.8±49.7 µg m^{-2} day^{-1}, respectively. The fluxes of dry and wet deposition samples were generally higher in Izmir than the values previously reported at different countries around the world.

The annual dry deposition fluxes of Cr, Cd, Pb, Cu, Zn and Ni were calculated as 32.2±23.1, 13.8±6.7, 30.1±13.6, 27.2±16.2, 710.5±217.6, and 46.6±21.3 kg km^{-2} yr^{-1}, respectively. Cr, Cd, Pb, Cu, Zn and Ni annual wet deposition fluxes were 6.9±3.5, 1.2±0.6, 2.7±1.7, 8.0±10.1, 74.9±91.1, and 2.7±1.1 kg km^{-2} yr^{-1}, respectively. The ratios of dry versus wet deposition per year were 4.7 times for Cr, 11.5 times for Cd, 11.1 times for Pb, 3.4 times for Cu, 9.5 times for Zn, and 17.3 times for Ni. Wet deposition rates are found to be more significant than the dry deposition rates on daily basis except Ni. However, dry deposition was more important than that of wet deposition throughout the study period in this suburban area.

In addition LUMIStox® toxicity test was used to determine the overall toxicity of the collected deposition samples. Toxicity evaluations are based on soluble fractions of the studied heavy metals. Overall toxicity levels in the environmental samples were compared with the individual toxicities due to the selected metals. Toxicity ranking of metals from most toxic to least toxic in this study was found as Cr>Cd>Pb>Cu>Zn>Ni. Good agreement was found for studied heavy metals between our results and most of the reported work of others except chromium toxicity level.

Rainwater samples were found to be 15% more toxic than dry deposition samples. Zinc was lead in dry deposition samples, while chromium was lead most of the rainwater samples. The studied metals are ready to impose ecotoxic impacts in the water-soil environments and on biota in relation to the soluble fraction ratios in deposition in Izmir.

Keywords: Atmospheric deposition, dry deposition, wet deposition, Mediterranean climate, heavy metals, air pollution, ecotoxicity, LUMIStox® toxicity test.

ATMOSFERİK ÇÖKELMENİN EKOTOKSİSİTESİ

ÖZ

Kuru ve ıslak çökelme örnekleri, Ekim 2003 ve Haziran 2004 döneminde Buca, İzmir'de toplanmıştır. Seçilen hava kirleticilerin (Cr, Cd, Pb, Cu, Zn ve Ni) kuru ve ıslak çökelmeleri, Su Yüzey Örnekleyicisi (WSS) kullanılarak ölçülmüştür. Kuru çökelme örnekleri 24 saatlik dönemler şeklinde, ıslak çökelme örnekleri ise yağışlı dönemlerde alınmıştır.

Kuru ve ıslak çökelme örnekleri filtrelenmiş ve filtreler ve süzüntüler, seçilen ağır metaller için grafit fırınlı atomik absorbsiyon spektrofotometresi kullanılarak analiz edilmiştir. Ancak Zn alevli teknik kullanılarak analiz edilmiştir.

Islak çökelme örnekleri için Cr, Cd, Pb, Cu, Zn ve Ni'in hacim ağırlıklı ortalama toplam ağır metal konsantrasyonları sırasıyla 17.1±8.5, 3.1±1.6, 6.6±4.1, 19.5±24.8, 184.2±224.0 ve 6.7±2.6 in µg L^{-1} olarak bulunmuştur. İzmir'de ölçülen bu konsantrasyonlar, daha önce dünyanın çeşitli yerlerinde ölçülmüş olan değerlerden daha yüksek bulunmuştur.

Cr, Cd, Pb, Cu, Zn ve Ni'in ortalama toplam kuru çökelme akıları sırasıyla 96.4±69.2, 41.3±20.0, 90.3±40.8, 81.3±48.6, 2127.2±651.4 ve 139.6±62.0 µg m^{-2} $gün^{-1}$'dür. Cr, Cd, Pb, Cu, Zn ve Ni'in ortalama toplam ıslak çökelme akıları ise sırasıyla 271.2±148.1, 54.3±46.1, 111.5±77.9, 362.5±670.5, 2387.7±2807.2 ve 107.8±49.7 µg m^{-2} $gün^{-1}$'dür. İzmir'de belirlenen kuru ve ıslak çökelme örneklerinin akıları, daha önce dünyanın çeşitli ülkeleri için belirlenmiş olan değerlerden daha yüksek bulunmuştur.

Cr, Cd, Pb, Cu, Zn ve Ni'in yıllık kuru çökelme akıları sırasıyla 32.2±23.1, 13.8±6.7, 30.1±13.6, 27.2±16.2, 710.5±217.6 ve 46.6±21.3 kg km^{-2} $yıl^{-1}$ olarak hesaplanmıştır. Cr, Cd, Pb, Cu, Zn ve Ni'in yıllık ıslak çökelme akıları ise sırasıyla 6.9±3.5, 1.2±0.6, 2.7±1.7, 8.0±10.1, 74.9±91.1 ve 2.7±1.1 kg km^{-2} $yıl^{-1}$'dır. Kuru çökelmenin yıllık bazda ıslak çökelmeye oranı Cr, Cd, Pb, Cu, Zn ve Ni için sırasıyla 4,7; 11,5; 11,1; 3,4; 9,5 ve 17,3 kez fazladır. Günlük bazda ıslak çökelme oranları, Ni dışında, kuru çökelme oranlarından daha önemli bulunmuştur. Bununla birlikte kuru çökelme, bu kent dışı alanda çalışma dönemi boyunca ıslak çökelmeden daha önemli olmuştur.

Toplanan çökelme örneklerinin toplam toksisitelerini belirlemek amacıyla LUMIStox® toksisite testi kullanılmıştır. Toksisite değerlendirmeleri, çalışılan ağır metallerin çözünmüş kısımlarında gerçekleştirilmiştir. Örneklerdeki toplam toksisite düzeyleri, seçilen ağır metallerin toksisiteleri ile karşılaştırılmıştır. Bu çalışmada metallerin toksisite sıralaması, en toksikten en az toksiğe doğru Cr>Cd>Pb>Cu>Zn>Ni şeklinde bulunmuştur. Çalışılan ağır metaller için bulduğumuz sonuçlar ile rapor edilen diğer çalışmaların çoğu arasında, krom toksisite düzeyi hariç olmak üzere iyi bir uyum bulunmuştur.

Yağmursuyu örnekleri, kuru çökelme örneklerinden %15 daha toksik bulunmuştur. Kuru çökelme örneklerinde Zn, ıslak çökelme örneklerinin çoğunda ise Cr baskın durumdadır. Çalışılan metaller, İzmir'deki çökelmede belirlenen çözünmüş kısım oranları ile ilişkili olarak, su-toprak ortamlarında ve canlı hayatı üzerinde, ekotoksik etkiler yapmaya hazır durumdadır.

Anahtar sözcükler: Atmosferik çökelme, kuru çökelme, ıslak çökelme, Akdeniz iklimi, ağır metaller, hava kirliliği, ekotoksisite, LUMIStox® toksisite testi.

CONTENTS

Page

THESIS EXAMINATION RESULT FORM ……………………………….ii
ACKNOWLEDGMENTS ……………………………………………….iii
ABSTRACT ……………………………………………………………..iv
ÖZ ……………………………………………………………………..vi

CHAPTER ONE – INTRODUCTION ……………………………………...1

CHAPTER TWO – LITERATURE REVIEW ……………………………..5

2.1 Heavy Metals in the Environment ……………………………………..5
  2.1.1 Sources of Heavy Metals in the Ambient Air ……………………..5
  2.1.2 Toxic Effects of Heavy Metals ……………………………………8

2.2 Heavy Metal Concentrations in Ambient Air …………………………14

2.3 Heavy Metal Concentrations in Rainwater ……………………………18

2.4 Atmospheric Deposition of Heavy Metals ……………………………21
  2.4.1 Dry Deposition of Heavy Metals ………………………………...28
  2.4.2 Wet Deposition of Heavy Metals ………………………………...32

2.5 Ecotoxicity and Test Methods ………………………………………...35
  2.5.1 Principles of Ecotoxicity …………………………………………35
  2.5.2 Methods for Ecotoxicity Tests …………………………………...36
  2.5.3 LUMIStox® Toxicity Test ………………………………………38

CHAPTER THREE – MATERIALS AND METHODS …………………45

3.1 Sampling …………………………………………………………45
  3.1.1 Sampling Location ……………………………………………45
  3.1.2 Sampling Program ……………………………………………46
  3.1.3 Sampling Methods ……………………………………………47
  3.1.4 Sample Handling and Preparation ……………………………50
  3.1.5 Preparation of Synthetic Heavy Metal Solutions ………………..52
    3.1.5.1 Single Synthetic Heavy Metal Solutions …………………...52
    3.1.5.2 Mixture Synthetic Heavy Metal Solutions …………………53

3.2 Heavy Metal Analysis ……………………………………………...53
  3.2.1 Extraction ……………………………………………………..53
  3.2.2 Analysis ………………………………………………………54

3.3 Quality Assurance …………………………………………………55

3.4 Toxicity Tests ……………………………………………………...58

3.5 Calculations ………………………………………………………..60
  3.5.1 Calculation of Heavy Metal Concentrations ……………………60
  3.5.2 Calculation of Dry and Wet Deposition of Heavy Metals ………60
  3.5.3 Calculation of EC_{50} Values of Single Heavy Metals ……………62
  3.5.4 Calculation of $EC_{50total}$ Values of Synthetic Metal Mixtures and Samples …………………………………………………64
  3.5.5 Calculation of Toxicity Index Values of Synthetic Metal Mixtures and Samples …………………………………………..66
  3.5.6 Calculation of Interactive Toxicity Effects of Synthetic Metal Mixtures ……………………………………………………...66

3.6 Statistical Evaluation ………………………………………………....67

CHAPTER FOUR – RESULTS AND DISCUSSION ……………………69

4.1 Heavy Metal Concentrations in Blank and Wet Deposition Samples ..69
    4.1.1 Heavy Metal Concentrations in Blank Samples ………………...69
    4.1.2 Heavy Metal Concentrations of Rainwater (Wet Deposition) Samples ……………………………………………………71
        4.1.2.1 Discussion of Metal Ion Concentrations in Wet Deposition Samples……..………………………………………........75
    4.1.3 Dissolved and Suspended Fractions of Environmental Samples 78

4.2 Dry and Wet Deposition Fluxes of Environmental Samples …………85
    4.2.1 Dry Deposition Fluxes ……………………………………..85
    4.2.2 Wet Deposition Fluxes of Rainwater Samples ………………….90
    4.2.3 Annual Heavy Metal Deposition Fluxes in Dry and Wet Forms .97

4.3 Toxicity Evaluation of Heavy Metals in Deposition ………………..100
    4.3.1 Inhibition Values ………………………………………………100
        4.3.1.1 Inhibition Values of Simulated Single Heavy Metal Solutions …………………………………………………..100
        4.3.1.2 Inhibition Values of Synthetic Metal Mixtures …………...102
        4.3.1.3 Inhibition Values of Dry Deposition Samples 102
        4.3.1.4 Inhibition Values of Rainwater Samples …………………106
    4.3.2 Effective Concentrations of Deposited Heavy Metals in Dry and Wet Forms on the Toxicity of Receiving Waters …………109
        4.3.2.1 EC_{50} Values of Simulated Single Heavy Metal Solutions ..109
        4.3.2.2 $EC_{50total}$ Values of Synthetic Metal Mixtures ……………..112
        4.3.2.3 $EC_{50total}$ Values of Dry Deposition Samples ………………113
        4.3.2.4 $EC_{50total}$ Values of Rainwater Samples ……………………114
    4.3.3 Toxicity Index …………………………………………………116
        4.3.3.1 Toxicity Index Values of Heavy Metals in Synthetic Metal Mixtures …………………………………………...116

4.3.3.2 Toxicity Index Values of Heavy Metals in Dry Deposition Samples ………………………………………………...117
4.3.3.3 Toxicity Index Values of Heavy Metals in Rainwater Samples …………………………………………………117
4.3.3.4 Interactive Toxicity Effects of Metal Mixtures …………...119

CHAPTER FIVE – CONCLUSIONS ………………..………………..120

5.1 Conclusions …………………………………………………………120
5.1.1 Concentrations of Aqueous Solutions Due To Wet Deposition...120
5.1.2 Dry Deposition Fluxes………………………………………..121
5.1.3 Wet Deposition Fluxes ………………………………………122
5.1.4 Comparison of Dry and Wet Deposition Fluxes ………………..123
5.1.5 Toxicity Evaluations …………………………………………123

5.2 Suggestions ………………………………………………………..125

REFERENCES …………………………………………………………126

CHAPTER ONE
INTRODUCTION

Metal transfer through the atmosphere is a significant part of the biogeochemical cycle of these elements. There are two processes which increase heavy metal concentrations in the atmosphere: natural and anthropogenic. Natural sources are mainly composed of soil, sea water and volcanic dusts and gases. Anthropogenic emissions come from industrial gases and aerosols or fossil-fuel combustion. Incineration of urban waste water treatment sludge and of urban waste was identified as major atmospheric sources of trace metals.

Atmospheric deposition occurs when these particles settle to the ground or water surfaces. Dry deposition occurs by direct impact and gravitational settling of discrete or aggregated particles onto land or water surfaces. In wet deposition, aerosols and gases are washed out and deposited either dissolved or suspended forms in water droplets or ice crystals. Besides such long-range transport processes, significant dry and wet depositions also occur locally, and atmospheric sources in urban area may play an important role in the metal contamination of dry and wet depositions.

The measurement of wet deposition is relatively straightforward; it involves the analysis of rainwater samples containing trace quantities of pollutants, at concentrations of $\mu g\ L^{-1}$ in aqueous solution. The measurement of dry deposition is more problematic (Azimi, Ludwig, Thevenot, & Colin, 2003). Recently, an aerodynamically designed water surface sampler (WSS) was used as the surrogate surface for direct measurement of dry deposition of heavy metals by Azimi et al. (2003), Golomb, Ryan, Eby, Underhill, & Zemba (1997), Morselli et al. (1999), and Sakata & Marumoto (2004).

Heavy metals constitute an important class of toxic inorganic elements. Examples of toxic heavy metals include cadmium, mercury, chromium, arsenic, barium, beryllium, nickel, selenium, silver, thallium, bromine and lead in elemental form or as several different compounds (U. S. Environmental Protection Agency [USEPA], 2004). High concentrations of airborne trace metals may seriously affect air quality, posing direct influences on human health and on the environmental media. As pollution-derived elements are often concentrated on fine particles, they could remain suspended in air with relatively long residence times and could efficiently penetrate human lungs. Characteristics of crustal versus anthropogenic particles containing heavy metal with respect to size distribution and types of salts involved affects their solubilities in water. This is turn is an important factor on their toxicities in water phase. Thus, trace metals associated with fine aerosol particles may contribute to aquatic toxicity in the environment. Since heavy metals present high toxicity and high lability in atmospheric fallout, their monitoring is important both in urban and rural areas.

Excess metal levels in surface water may pose health risks to humans and to the environment. Aquatic organisms may be adversely affected by heavy metals in the environment. The toxicity is largely a function of the water chemistry and sediment composition in the surface water system. Metal uptake rates will vary according to the organism and the metal in question. Phytoplankton and zooplankton often assimilate available metals quickly because of their high surface area to volume ratio. The ability of fish and invertebrates to adsorb metals is largely dependent on the physical and chemical characteristics of the metal (Watershedss, 2003).

Toxic heavy metals in the environment are evaluated by using different ecotoxicity tests. The LUMIStox® test which is one of these tests is a bioassay test for measuring the toxicity of environmental samples. LUMIStox® test was developed with a certain type of luminescent bacteria in conformity with DIN 38412 L34 and L341. The inhibitory effect on the luminescent bacteria was determined by a static test. Certain volumes of test substances were combined with suspension of luminescent bacteria in a cell and measured. This bioassay test enables the user to determine the toxicity of aqueous samples or sample extracts with the help of luminescent bacteria (Dr.Lange, 1994).

The specific objectives of this study were as follows:

1. To measure selected heavy metals (Cr, Cd, Pb, Cu, Zn and Ni) concentrations of dry and wet deposition samples obtained from WSS.

2. To determine the dry deposition fluxes of selected heavy metals using surrogate surfaces.

3. To determine the wet deposition fluxes of selected heavy metals.

4. To determine relative importance of dry and wet deposition fluxes in total (dry+wet) deposition.

5. To evaluate the relationships between heavy metal concentrations and fluxes of dry and wet deposition samples with the meteorological parameters during the sampling period.

6. To determine individual toxicity values (EC_{50}) of Cr, Cd, Pb, Cu, Zn and Ni.

7. To determine overall toxicity values ($EC_{50total}$) of synthetic metal mixtures.

8. To determine overall toxicity values ($EC_{50total}$) of dry and wet deposition samples.

9. To determine toxicity index (TI) values of synthetic metal mixtures dry and wet deposition samples.

10. To determine the interactive effects among heavy metals.

11. To evaluate the relationships between heavy metal concentrations with the toxicity values of dry and wet deposition samples.

To meet these objectives, a sampling program was carried out between October 2003 and June 2004 in Buca, Izmir. Dry and wet deposition samples were collected using a WSS and were analyzed to determine the concentrations of Cr, Cd, Pb, Cu, Zn and Ni using a Perkin-Elmer Model 700 atomic absorption spectrophotometer equipped with a graphite furnace except for zinc which was analyzed by using a flame technique. In addition LUMIStox® toxicity test was used to determine the overall toxicity of these collected deposition samples. Rainwater concentrations, dry and wet deposition fluxes, and toxicity values obtained from experimental studies were evaluated by comparing them to the values reported in previous studies.

This study consists of six chapters. An overview and the objectives of the study were presented in Chapter 1. Chapter 2 reviews the concepts and previous studies related to this work. Experimental work is summarized in Chapter 3. Results and discussions were presented in Chapter 4. Chapter 5 summarizes the conclusions and suggested future work.

CHAPTER TWO
LITERATURE REVIEW

In this thesis a thorough study is carried out with some heavy metals dissolving in environmental waters after deposition from the atmosphere where they are present as air pollutants. Ecotoxicity in the water environments due to deposition from the polluted air is within the scope of the study.

This chapter presents background information on chemical properties, sources, toxicities, ambient concentrations, and dry and wet deposition fluxes of heavy metals of concern reported in the literature. In addition to that, basic principles of the commonly used test methods for determining ecotoxicity are mentioned as well as the principles of LUMIStox® method and toxicity evaluations for heavy metals reported with this method are given in detail.

2.1 Heavy Metals in the Environment

2.1.1 Sources of Heavy Metals in the Ambient Air

Heavy metals enter the environment by way of two groups of mechanisms. One of them is the natural processes (including erosion of ore-bearing rocks, wind-blown dust, volcanic avtivity and forest fires). The second mechanism involves processes derived from human activities by means of atmospheric deposition, direct discharges or dumping into water bodies. For some metals such as Hg and Cd, natural and anthropogenic inputs are of the same order of magnitude; whilst for others (for example Pb) inputs due to human activities exceed the natural inputs (Prego, 2003).

Pb, Hg, Cd, As, Cr, Zn and Cu are widely used in industry, particularly in metal-working or metal-plating, and in such products as batteries and electronics. These metals also are used in the production of jewelry, paint pigments, pottery glazes, inks, dyes, rubber, plastics, pesticides, and even in medicines. Metals from these operations are released into the environment during production and usage (Landis & Yu, 1999).

Toxic air pollutants can be carried by the winds to areas far from the pollution source. The weather conditions, the terrain (i.e., mountains, plains, valleys), and the chemical and physical properties of the pollutants determine the transportation distances and the physical and chemical changes these pollutants may undergo. Some of the airborne pollutants can be deposited to land and water bodies through precipitation, or by settling directly onto land or water. Repeated cycles of transport, deposition, and evaporation can move toxic air pollutants to different environmental media. Kanellopoulou (2001) has reported that a large percentage of metals are settled by the rain at or near the places of their production while the fine particles are easily transferred by the wind and rained out at long distances from the point of their emission. Some heavy metals remain airborne for a long time; for example mercury and lead are of particular concern because they clear out from air very slowly or not at all (Mowat, 2000).

Chromium is a common contaminant in the environment. It occurs naturally in rocks, animals, plants, soil, and in volcanic dust and gases. Chromium is present in the environment in several different forms. The most common forms are elemental chromium(0) or chromium(III) and chromium(VI)salts. Cr(VI) and Cr(III) are used for chrome plating, dyes and pigments, leather tanning, and wood preserving (Agency for Toxic Substances and Disease Registry [ATSDR], 2001). Cr(VI) rarely occurs naturally such as in the mineral crocoite ($PbCrO_4$), but is produced from anthropogenic sources. The primary sources of hexavalent chromium Cr(VI) in the atmosphere are chromate chemicals used as rust inhibitors in cooling towers and emitted as mists, particulate matter emitted during manufacture and use of metal chromates, and chromic acid mist from the plating industry.

Hexavalent chromium in the air eventually reacts with dust particles or other pollutants to form trivalent chromium and then both hexavalent and trivalent chromium are removed from air by atmospheric fallout and precipitation. The atmospheric half-life for the physical removal mechanism is dependent on the particle size and particle density. Chromium particles of small aerodynamic diameter (< 10 µm) remain airborne for a long period (United States Environmental Protection Agency [USEPA], 1998).

Elemental cadmium is found naturally in the earth's crust. However, the most common forms of cadmium found in the environment exist in combinations with other elements (ATSDR, 1999). Atmospheric emission of cadmium may arise from such activities as mining and metallurgical processing, combustion of fossil fuel, textile printing, application of fertilizers and fungicides, recycling of ferrous scraps and motor oils, disposal and incineration of cadmium containing products (e.g., plastics), and tobacco smoke (Landis & Yu, 1999).

Lead is a natural element that persists in water and soil. Lead particles in the atmosphere have a residence time of about 10 days. Most of the lead in environmental media is of anthropogenic sources (The Risk Assessment Information System [RAIS], 2004a). Lead smelters, burning of coal and materials containing lead, refining of scrap, wind blown soil dust, and lead alkyls from gasoline are sources of atmospheric lead. Significant quantities of lead can be discharged from the smokestacks and other fugitive emission sources from smelters and refining processes into the air, soils, and onto the vegetation growing nearby (Landis & Yu, 1999).

Copper can enter the environment through releases from the mining of copper and other metals, and from factories that make or use copper metal or copper compounds. Copper can also enter the environment through domestic waste water, combustion of fossil fuels and wastes, wood production, phosphate fertilizer production, and natural sources (for example, windblown dust, from native soils, volcanoes, decaying vegetation, forest fires, and sea spray) (ATSDR, 2004).

Zinc is released into the environment by natural processes, human activities like mining, steel production, coal burning, and burning of waste (ATSDR, 2005c). Other sources of zinc along with several other metals are coal-fire power plant emissions, incinerator off-gases, vehicle exhausts, and urban road dust (Paode et al., 1998). Zinc combines with other elements to form zinc compounds. Common zinc compounds found at hazardous waste sites include zinc chloride, zinc oxide, zinc sulfate, and zinc sulfide. Zinc has many commercial uses as coatings to prevent rust, in dry cell batteries, and mixed with other metals to make alloys like brass, and bronze (ATSDR, 2005c).

Nickel is a naturally occurring element and may exist in various mineral forms (RAIS, 2004b). Nickel is emitted from volcanoes. Nickel is also found in meteorites and on the ocean floor (ATSDR, 2005b). Nickel is used in a wide variety of applications including metallurgical processes and electrical components (such as batteries) (RAIS, 2004b). Anthropogenic sources of nickel are burning of fossil fuels, municipal wastes, and nickel manufacturing (Mowat, 2000).

2.1.2 Toxic Effects of Heavy Metals

Toxic air pollutants, also known as hazardous air pollutants, are with known or suspected carcinogenic activity or other serious health effects, such as reproductive effects or birth defects, or adverse environmental effects. These toxic air pollutants are classified as inorganic or organic “air toxics”. Heavy metals constitute an important class of toxic inorganic elements. Examples of toxic air pollutants include several metals such as cadmium, mercury, chromium, arsenic, barium, beryllium, nickel, selenium, silver, thallium, bromine and lead in elemental form or as several different compounds (USEPA, 2004).

People exposed to toxic air pollutants at sufficient concentrations and durations may have an increased chance of getting cancer or experiencing other serious health effects. These health effects may include damage to the immune system, as well as neurological, reproductive (e.g., reduced fertility), developmental, respiratory and other health problems. In addition to exposure from breathing air toxics, some toxic air pollutants can deposit onto soils or surface waters, where they are taken up by plants and ingested by animals and are eventually magnified in the food chain. Like humans, animals may experience health problems if exposed to sufficient quantities of air toxics over time (USEPA, 2004).

Wherever metals are extracted or processed, particles of metallic dust are scattered into the air. Rusting and other forms of corrosion continue the spread of metals once products containing metals have been brought into use, and when they later end up at a scrap yard or landfill site. The burning of fossil fuels, biomass fuels or waste also releases metals, which then enter the atmosphere. The heaviest deposition of airborne metal particles occurs in the vicinity of mines, smelters and metal processing/heavy engineering works, which constitute the main emission sources. Many of the particles are so small that they can be carried enormous distances by the wind. But the bulk of the metals emitted into the air over the years remain in the ground where they deposited. Concentrations of heavy metals in the soil close to some large metal processing/engineering works are so high that they hamper the ability of microorganisms to break down plant matter, thus also impeding the release of nutrients from this material (Swedish Environmental Protection Agency [Swedish EPA], 2001).

All heavy metals exist in surface waters in colloidal, particulate, and dissolved phases. The colloidal and particulate metal may be found in hydroxides, oxides, silicates, or sulfides; or adsorbed to clay, silica, or organic matter. The soluble forms are generally ions or unionized organometallic chelates or complexes. The behavior of metals in natural waters is a function of the substrate sediment composition, the suspended sediment composition, and the water chemistry. Sediments composed of fine sand and silt will generally have higher levels of adsorbed metals than the sediments with quartz, feldspar, and detrital carbonate-rich ingredients. Metals also have a high affinity for humic acids, organo-clays, and oxides coated with organic matter (Watershedss, 2003).

Water transports dissolved metals. Although dissolved metals are primarily transported in overland flow, some underground transport is possible. Metals that are introduced to the unsaturated zone and the saturated zone will most likely not be transported a long distance. Dissolved metals that are carried below the land surface will readily sorbs to soil particles or lithic material in the unsaturated zone and the saturated zone. Metals introduced into the atmosphere may be carried to the land surface by precipitation and dry fallout. Additionally, because metals readily sorp to many sediment types, wind-borne sediment is a potential route for metal transport. The water chemistry of the system controls the rate of adsorption and desorption of metals to and from sediment. Adsorption removes the metal from the water column and stores the metal in the substrate. Desorption returns the metal to the water column, where recirculation and bio-assimilation may take place. Metals may be desorbed from the sediment if the water experiences increases in salinity, decreases in redox potential, or decreases in pH (Watersheds, 2003).

Metals may enter the systems of aquatic organisms via three main pathways: 1) Free metal ions that are absorbed through respiratory surface are readily diffused into the blood stream. 2) Free metal ions that are adsorbed onto body surfaces are passively diffused into the blood stream. 3) Metals that are sorbed onto food and particulates may be ingested, as well as free ions ingested with water. Slightly elevated metal levels in natural waters may cause the following sub-lethal effects in aquatic organisms: 1) histological or morphological change in tissues; 2) changes in physiology, such as suppression of growth and development, poor swimming performance, changes in circulation; 3) change in biochemistry, such as enzyme activity and blood chemistry; 4) change in behavior; 5) and changes in reproduction (Watershedss, 2003).

Metals play an important role in cellular physiology, primarily through interactions with proteins including enzymes. Metals also interact with other biological molecules such as nucleic acids and lipids. Some metals are an integral part of enzymes and the activities of enzymes depend on the presence of the metals. Metals also alter the secondary and tertiary or the quaternary structure of proteins which leads to the stabilization of protein structure (Ren & Frymier, 2003). After absorption, these metals can bind to vital cellular components such as structural proteins, enzymes, and nucleic acids, and interfere with their functioning (Landis & Yu, 1999).

Only trace amounts of heavy metals are necessary for optimal cellular function and an over-dose results in toxicity. Some of these metals can cause severe physiological and health effects even in small amounts (Landis & Yu, 1999). The toxic effect of heavy metals in microorganisms depends on reactions with ligands that are essential for the normal physiological functions (Ren & Frymier, 2003). Each metal has a primary effect seen in a specific organ or tissue and most metals affect multiple organ systems. Klaasen (1996) explained that although certain metal ions were essential for biological activity in one concentration range, the same metals can become toxic at other concentrations, thus exhibiting the classic dose-response behavior. The degree to which a toxic pollutant affects a person's health depends on the quantity of pollutant the person is exposed to, the duration and frequency of exposure, the toxicity of the chemical, and the person's state of health and susceptibility.

Hexavalent chromium is in general more toxic to organisms in the environment that the trivalent chromium. Almost all the hexavalent chromium in the environment is a result of human activities. In this oxidation state, chromium is relatively stable in air and pure water, but it is reduced to the trivalent state when it comes into contact with organic matter in biota, soil, and water. The main features are inhibition of growth and inhibition of various metabolic processes such as photosynthesis or protein synthesis. Hexavalent chromium is accumulated by aquatic species by passive diffusion. Several factors affect the availability of chromium for the plant, including the pH of the soil, interactions with other minerals or organic chelating compounds, and carbon dioxide and oxygen concentrations. Little chromium is translocated from the site of absorption; however, the chelated form is transported throughout the plant (European Commission Report, 2002).

In air, chromium compounds are present mostly as fine dust particles which eventually settle over land and water. Chromium can strongly attach to soil and only a small amount can dissolve in water and move deeper in the soil to underground water (ATSDR, 2001). Hexavalent chromium may exist in aquatic media as water soluble complex anions and may persist in water. It also may react with organic matter or other reducing agents to form trivalent chromium. Hexavalent chromium in soil tends to be reduced to trivalent chromium by organic matter (USEPA, 1998).

Cadmium compounds are often found in or attached to the small particulate matter in the respirable range (diameter 0.1-1 µm) in the air. Cadmium can enter the air and travel a long way before coming down to earth as dust, or in rain or snow with these small particles. The residence time of cadmium in the air is relatively short (days to weeks) but sufficient to allow long-range transport in the atmosphere (World Health Organization [WHO], 2000).

Cadmium is readily accumulated by many organisms, particularly by microorganisms and mollusks. Soil invertebrates also concentrate cadmium markedly. The most affected soil microorganisms are fungi, some species being eliminated after exposure to cadmium in soil (European Commission Report, 2002). Cadmium is fairly mobile in soil and become even more mobile with falling pH levels. Continuing soil acidification therefore involves a risk of rising cadmium concentration in nearby waters (Swedish EPA, 2001).

Cadmium is toxic to a wide range of microorganisms. The main effect is on growth and replication. In aquatic systems, cadmium is most readily absorbed by organisms directly from the water in its free ionic form Cd (II). The acute toxicity of cadmium to aquatic organisms is variable, even between closely related species, and is related to the free ionic concentration of the metal. Stomatal opening, transpiration, and photosynthesis have been reported to be affected by cadmium in nutrient solutions, but metal is taken up into plants more readily from nutrient solutions than from soil. Terrestrial plants may accumulate cadmium in the roots and cadmium is found bound to the cell walls. Cadmium significantly influences leaf litter decomposition (European Commission Report, 2002).

The pathway of human exposure from agricultural crops is susceptible to increases in soil cadmium as increase in soil cadmium contents, e.g. due to cadmium in soil amendment products, result in an increase in the uptake of cadmium by plants (European Commission Report, 2002).

When lead is released into the atmosphere, it may travel long distances before settling to the ground. Once lead falls onto soil, it usually sticks to soil particles. Movement of lead from soil into groundwater will depend on the type of lead compound and the characteristics of the soil (ATSDR, 2005a). Lead, in particular, is very easily fixed in the more layer of soil and only migrates out very slow. Even though we have now seen a dramatic decrease in lead deposition, it appears likely that the concentrations of lead in soil remain strongly elevated (Swedish EPA, 2001).

Lead binds strongly to particles, such as soil, sediment and sewage sludge in the environment. Lead tends to precipitate out of complex solutions. It does not bio-accumulate in most organisms, but can accumulate in biota feeding primarily on particles, e.g. mussels and worms. These organisms often possess special metal binding proteins that remove the metals from general distribution in their organism. Lead is taken up by terrestrial plants through the roots and to a lesser extent through the shoots. Translocation of the ion in plants is limited and most bound lead stays at root or leaf surfaces (European Commission Report, 2002).

Copper released into the environment usually attaches to particles made of organic matter, clay, soil, or sand. When copper is released into soil, it typically becomes strongly attached to the organic material and minerals in the top layers of soil and does not move very far when it is released. When copper is released into water, the copper that dissolves can be carried in surface waters either as free copper or, more likely, bound to particles suspended in the water. Because copper binds so strongly to suspended particles and sediments, it typically does not enter groundwater. Copper that enters water eventually collects in the sediments of rivers, lakes, and estuaries. Copper is carried on particles, and is then carried back to earth through gravity or in rain or snow (ATSDR, 2004).

Zinc attaches to soil, sediments, and dust particles in the air. Rain and snow remove zinc dust particles from the air. Depending on the type of soil, some zinc compounds can move into the groundwater and into lakes, streams, and rivers. Most of the zinc in soil stays bound to soil particles and does not dissolve in water. It builds up in fish and other organisms, but it does not build up in plants (ATSDR, 2005c).

In the air, nickel attaches to small particles that settle to the ground or are taken out of the air in rain or snow; this usually takes many days. Nickel released in industrial wastewater ends up in soil or sediment where it strongly attaches to particles containing iron or manganese (ATSDR, 2005b).

2.2 Heavy Metal Concentrations in Ambient Air

Ambient air concentrations of heavy metals have been measured in different places around the world. Although there are numerous studies on ambient heavy metal concentrations, they differ greatly from each other in terms of effects of local heavy metal sources, sampling method, sampling duration, sample preparation, and analysis method.

Prevention of the environmental pollution by heavy metals is a topical problem various aspects of which are studied by individual researchers and different national and international organizations. For example the Convention on Long-Range Transboundary Air Pollution Cooperative Programme for Monitoring and Evaluation of the Long-Range Transmission of Air Pollutants in Europe (EMEP) which was signed in 1998 aimed at the control of atmospheric emissions of heavy metals. According to this protocol lead, cadmium and mercury have the first priority. Measurements of heavy metal concentrations in air and precipitation were carried out on EMEP monitoring network for the period from 1990 to 2001. The regional and hemispheric transport models were used for evaluation of the heavy metal pollution levels in Europe and in the Northern Hemisphere. In the scope of this protocol, the concentrations of lead and cadmium were determined as 8.8 and 0.22 ng m^{-3}, respectively for Turkey in 2001. The total anthropogenic emissions of lead and cadmium were presented as 774.0 and 14.0 t yr^{-1}, respectively (EMEP Report, 2003).

Ambient air concentrations of heavy metals were measured in different cities in Turkey (Alp, Çitil, Eldem, & Bayhan, 1999; Örnektekin, Mıstıkoğlu, & Özyılmaz, 1999). Several studies were carried out to determine the air quality levels in Izmir city with appreciable air pollution due to industrial facilities. Major industries include cement, petroleum, and metal smelting. In addition to them foundries and transportation are important activities. Several studies on heavy metals were carried out in the Aliaga Industrial Region (Bayram, Yılmaz, Odabasi, & Muezzinoglu,1997; Türkan, Henden, Çelik, & Kıvılcım, 1995). All of these studies showed that Izmir region is heavily polluted with heavy metals.

Significance of heavy metal pollution differs between rural, urban and suburban areas and with respect to proximity to industrial areas. The average trace element concentrations according to Bozlaker (2002) Pb, Cr, Zn, Cd, Cu, Ni, Mn, Mg, Al, Ca and Fe measured in the ambient aerosols and dry deposition samples at the same site in this research in Izmir, Turkey were generally higher than those reported previously for other urban and rural areas in the world. Even higher Cu, Zn and Fe concentrations were measured previously at Aliaga sampling sites located around the numerous steel plants at 45 km northwest of Izmir (Bayram et al., 1997). Taşdemir & Kural (2005) have reported that anthropogenic sources generate the significant components of trace elements in the urban atmosphere in Bursa. Concentrations of the metals measured in suburban area, Uludağ were found lower than those in urban area, Bursa, Turkey by Samura, Al-Agha, & Tuncel (2003). Zn, Cu and Cd levels in total suspended particles and airborne particulate trace metals were also found high in comparison with other urban and industrial areas in the Baixada Fluminense, in Brazil by Quiterio, Sousa, Arbilla, & Escaleira (2005).

A significant seasonal variation was not observed although trace elements showed similar temporal variability in the study made by Bozlaker (2002). This is in contrast to the results of the study was made by Samura et al. (2003) who reported that the concentrations of crustal elements were found higher in summer than winter, while anthropogenic elements had higher concentrations in winter than summer in Bursa, Turkey. Taşdemir & Kural (2005) have reported that residental heating was partially considered as a trace element source during spring months in urban areas in Bursa.

Gao et al. (2002) have reported that the ambient concentrations of trace elements at a specific location are largely dependent upon the distance from their sources.

Ambient air particulate-phase heavy metal concentrations measured in different places around the world are summarized in Table 2.1.

Table 2.1 Particulate trace metal concentrations in air around the world (ng m^{-3})

Concentration						**Sampling Method**	**Location**	**Period**	**Reference**
Cr	**Cd**	**Pb**	**Cu**	**Zn**	**Ni**				
10.7	0.7	77.2	396.6	250.5	5.1	High-volume air sampler	Bursa, Turkey	March 2003 to June 2003	Tasdemir et al., in press
-	0.61	22.7	17.1	96.2	-	High-volume air sampler	Komae, Tokyo	May 2002 to December 2002	Sakata & Marumoto, 2004
-	-	180.0	240.0	960.0	-	TSP sampler	Sha-Lu, Taiwan	August 2003 to September 2003	Fang et al., 2004
0.50	0.20	5.3	1.5	13.0	1.5	Filter pack sampler	Denmark	In 2002	Ellermann et al., 2003
10.5	8.4	110.8	154.2	733.4	39.0	High-volume air sampler	Izmir, Turkey	September 2000 and June 2001	Bozlaker, 2002
1.8	0.21	6.5	9.7	21.0	6.0	Low-volume air sampler	New York-New Jersey Harbor Bight, USA	January 1998 to January 1999	Gao et al., 2002
-	0.21	43.7	9.6	-	1.4	High-volume air sampler	Seville, Spain	In the spring of 1996	Espinosa et al., 2002
-	1.0	83.0	210.0	112.0	5.0	High-volume air sampler	Huelva, Spain	July 1999 to January 2001	Querol et al., 2002
13.0	2.0	60.0	58.0	304.0	5.0	Low-volume air sampler	Tito Scalo, Italy	April 1997 to September 1999	Ragosta et al., 2002
6.0	-	149.0	74.0	250.0	7.0	High-volume air sampler	Barcelona, Spain	June 1999 to June 2000	Querol et al., 2001
2.4	0.24	34.2	5.7	89.0	-	High-volume air sampler	Tel-Shikmona, Israel	Between 1994 and 1997	Herut et al., 2001
4.2	-	48.4	18.7	135.8	-	Dichotomous PM$_{10}$ sampler	Chicago, USA	December 1993 to October 1995	Yi et al., 2001a
12.0	0.39	49.0	-	84.0	-	Low pressure impactor	Burnaby Lake, Canada	A period of 18 weeks in 1995	Brewer & Belzer, 2001
-	0.03	1.4	-	0.78	0.40	Modeled	Russian Arctic	1986 to 1995	Vinogradova, 2000
42.9	3.3	510.0	260.0	-	17.4	High-volume air sampler	Istanbul, Turkey	08 July 1997 to 21 July 1997	Alp et al., 1999
260.0	50.0	760.0	1520.0	-	790.0	Low-volume air sampler	Iskenderun Bay, Turkey	May 1998 to March 1999	Örnektekin et al., 1999

2.3 Heavy Metal Concentrations in Rainwater

Several studies were performed to determine the concentrations of heavy metals in rainwater in Turkey and other countries around the world. Some researchers reported seasonal variations in heavy metal concentrations in rainwaters whilst in some other studies such variations were not notable. Kaya & Tuncel (1997) reported that concentrations of crustal elements and ions are higher during summer season, while concentrations of anthropogenic ions and elements did not show well defined seasonal cycles in precipitation in Ankara. Concentrations of most of the elements in rainwater were found higher in summer than winter in Istanbul, Turkey (Basak & Alagha, 2004). Conko & Rice (2000) reported that seasonal trends in volume weighted concentrations were observed for most elements, with a summer maximum corresponding to the peak acidity in regional precipitation in Northern Virginia, U.S.A. A study made by Kanellopoulou (2001) has shown that low heavy metal concentrations were observed during the cold period October 1997 to March 1998 in Athens University Campus, Greece.

Along with seasonal variability of the heavy metal concentrations in rainwater, strong spatial variations have been identified. Especially that the heavy metal concentrations in rainwater in urban and industrial areas are much higher than rural areas. This may be explained by the ease of transport from emission sources and the presence of multiple sources. For example a study made by Örnektekin & Çakmaklı (2003) indicated highly variable metal ions concentrations in rainwater in Iskenderun, Turkey. Concentrations of Ca and Fe ions were found higher in the industrial zone and Payas city center. In the other three stations, concentrations of metal ions and NO_3^- ion were found lower than that of industrial zone. Deboudt, Flament, & Bertho (2004) have reported that the high variability was observed in heavy metal concentrations associated with rainwater samples collected in urban and industrial areas in the Eastern Channel (Northern France).

Long range transport can be proven by source apportionment modeling techniques or by using air-mass trajectory models. Alagha & Tuncel (2003) have shown that a measurement station in Amasra, Turkey near the Black Sea region received different amounts and types of anthropogenic pollutants via long range transport by using trajectory models. However, Halstead, Cunninghame, & Hunter (2000) have reported trace metal concentrations in the rainwater samples collected in Fiordland, New Zealand were not obviously related to air-mass trajectories.

Heavy metal concentrations in rainwater samples determined in different countries are shown in Table 2.2.

Table 2.2 Heavy metals concentrations in rainwater samples around the world (µg L^{-1})

Concentration						Sampling Method	Location	Period	Reference
Cr	**Cd**	**Pb**	**Cu**	**Zn**	**Ni**				
17.1	3.1	6.6	19.5	184.2	6.7	Water surface sampler	Izmir, Turkey	October 2003 to June 2004	Muezzinoglu & C.Cizmecioglu (in press)
-	0.20	8.1	4.3	30.0	3.4	Precipitation collector	Eastern Channel, France	September 1995 to June 1996	Deboudt et al., 2004
1.6	0.33	3.4	5.6	7.2	3.9	Wet-only sampler	Singapore	In 2000	Hu & Balasubramanian, 2003
-	1.5	102.0	-	-	9.0	A bottle fitted with a polythene funnel	London, England	In 1990	Nouri et al., 2001
1.3	0.20	0.88	15.4	33.5	4.1	-	Athens, Greece	Octomber 1997 to March 1998	Kanellopoulou, 2001
-	0.063	1.2	0.62	4.8	0.26	Wet-only precipitation sampler	Higashi–Hiroshima, Japan	1995-1997	Takeda et al., 2000
-	22.0	37.0	37.0	143.0	-	Rain collector	Amman, Jordan	October 1996 to April 1997	Jaradat et al., 1999
-	0.20	8.1	4.3	30.0	3.4	Rain collector	French coastal zone, France	December 1993 to February 1996	Maneux et al., 1999
3.0	9.5	19.1	6.1	0.03	4.1	Anderson acid precipitation sampler	Ankara, Turkey	November 1992 to July 1994	Kaya & Tuncel, 1997

2.4 Atmospheric Deposition of Heavy Metals

Atmosphere is an important environmental compartment in the biogeochemical cycles of trace metals (Hu & Balasubramanian, 2003). Atmospheric deposition is an important pathway for the transfer of pollutants from the atmosphere to water and soil environments (Shannigrahi, Fukushima, & Ozaki, 2005). Atmospheric deposition of particles to ecosystems may take place via wet and dry processes: wet deposition (by precipitation scavenging in which particles are deposited in rain and snow), the dry deposition which is much slower, and occult deposition (by fog, cloud-water, and mist interception) (Grantz, Garner, & Johnson, 2003).

Atmospheric deposition is considered to be a major source of toxic metals such as Hg, Cd, and Pb and other trace metals to ecosystems. In atmospheric droplets, trace metals such as Fe, Mn, and Cu have been implicated in the catalysis of SO_2 oxidation, leading to enhanced acidity of hydrometeors. Certain trace metals, emitted from particular source types, can be used to help identify the origin of the precipitating air mass and the sources from which the acid precipitation is derived (Hu & Balasubramanian, 2003).

In the eastern Mediterranean areas the major ion composition of wet and dry deposition were studied by Al-Momani, Aygun, & Tuncel (1998). Although they did not study the heavy metals in the cited work, these authors indicated that dry deposition is generally a more important mechanism due to the prevalence of marine and crustal ions which have larger particle sizes and also in view of the relative scarcity of rain events in this part of the world. For secondary particles containing NO_3^-, NH_4^+ or (non-sea salt) SO_4^{2-} however, their data showed the relative importance of wet deposition due to the small size aerosol formation originating from atmospheric gas to particle conversion mechanisms. It is indicated by Grantz et al. (2003) that dry deposition is most effective for coarse (primary) particles containing Fe and Mn whilst wet deposition has been found to be effective for fine particles of secondary origin and for elements such as Cr, Cd, Pb, Ni, V. Sweet, Weiss, & Vermette (1998) also reports that dry deposition fluxes are controlled by the concentration of trace metals in large particles.

It is necessary to measure both wet and dry deposition to obtain total atmospheric loading (Golomb et al., 1997). Although wet deposition is found to be more effective to remove the metals from the air by several researchers (Grömping, Ostapczuk, & Emons, 1997; Tasić, Rajić, & Novaković, 2001), availability of precipitation is another factor to take into account in Mediterranean climate areas. Therefore, the relative importance of wet versus the dry deposition may change not only on the basis of comparative efficiencies of the two mechanisms, but also with the local climatic properties. In the Mediterranean areas having climates with elongated dry periods, dry deposition is the main mechanism of the atmospheric cleansing.

Sakata, Marumoto, Narukawa, & Asakura (2006) have reported that the wet deposition fluxes of Hg, Pb and Se exceeds the dry deposition fluxes at most sites in Japan. In contrast, the dry deposition fluxes of Cr, Cu, Mn, Mo, Ni and V were found significantly higher than their wet deposition fluxes. Regional variations were observed between dry and wet deposition fluxes of trace elements, too. Highest dry deposition fluxes were observed in industrial and urban areas. However, from presented results of a study by Tasić et al. (2001), it can be concluded that wet deposition was the predominant removal process for heavy metals in sub-urban area of Belgrade-Zemun. Atmospheric deposition fluxes at urban area were higher than the fluxes of rural area demonstrating the impact of anthropogenic activities in Paris, France by Azimi et al. (2003).

Likewise, the highest values of atmospheric deposition fluxes were observed in Paris centre (Azimi, Rocher, Muller, Moilleron, & Thevenot, 2005). The seasonal distribution of these pollutants suggested the increase of dust loads containing heavy metals during summer. Wong, Li, Zhang, Qi, & Peng (2003) have reported that atmospheric deposition of Cu, Cr and Zn was generally higher in the summer than in winter, which could be due to the washout effect of the rainy season in the subtropical region in Pearl River Delta, China.

The study made at three Integrated Atmospheric Deposition Network (IADN) monitoring stations on Lakes Superior, Michigan and Erie, USA showed that both wet and dry deposition made an important contribution to the total atmospheric flux of trace metal deposition. Total particle mass concentrations were higher during the summer and fall at the Lake Erie site, however no seasonal trends in total particle mass at the other sites or trace metals at any of the sites were detected (Sweet et al., 1998).

Deposition levels of Pb, Cd ve Hg were determined the EMEP Report (2003) prepared by Ilyin et al. Spatial distribution of Pb deposition was more or less uniform in Europe (Figure 2.1). Deposition fluxes of Pb were found range from 0.5 to 1.5 kg km^{-2} yr^{-1} on most part of the European territory in 2001. However, they have explained that significantly higher Pb deposition values were obtained in a number of countries (at some places more than 10 kg km^{-2} yr^{-1}). Most part of the European area was characterized by Cd deposition intensity ranging between 0.010-0.050 kg km^{-2} yr^{-1} in 2001 (Figure 2.2). High Cd fluxes were characteristic in some countries such as in Central Europe, Italy and Russia. The lowest values of atmospheric Cd depositions were obtained for Northern Europe (EMEP Report, 2003).

A note is prepared for Turkey which is a contributor of EMEP project with country-oriented information on transboundary pollution by heavy metals and persistent organic pollutants. In the scope of this note, trends of emissions, atmospheric concentrations and depositions of Pb, Cd and Hg for the period from 1990 to 2001 were evaluated and spatial distribution of emissions and fluxes were determined for 2001. Pb deposition levels have indicated the regional variations in Turkey as shown in Figure 2.3. Minimum and maximum Pb deposition fluxes were found as 0.038 and 9.7 kg km^{-2} yr^{-1}, respectively in 2001. In a number of cities Pb deposition flux values had a range of 0.38-2.4 kg km^{-2} yr^{-1}. Minimum and maximum Cd deposition fluxes were 0.23 and 616 g km^{-2} yr^{-1}, respectively in 2001 (Figure 2.4). Most part of Turkey was characterized by Cd deposition intensities ranging between 1.7-86 g km^{-2} yr^{-1} (EMEP Note, 2003).

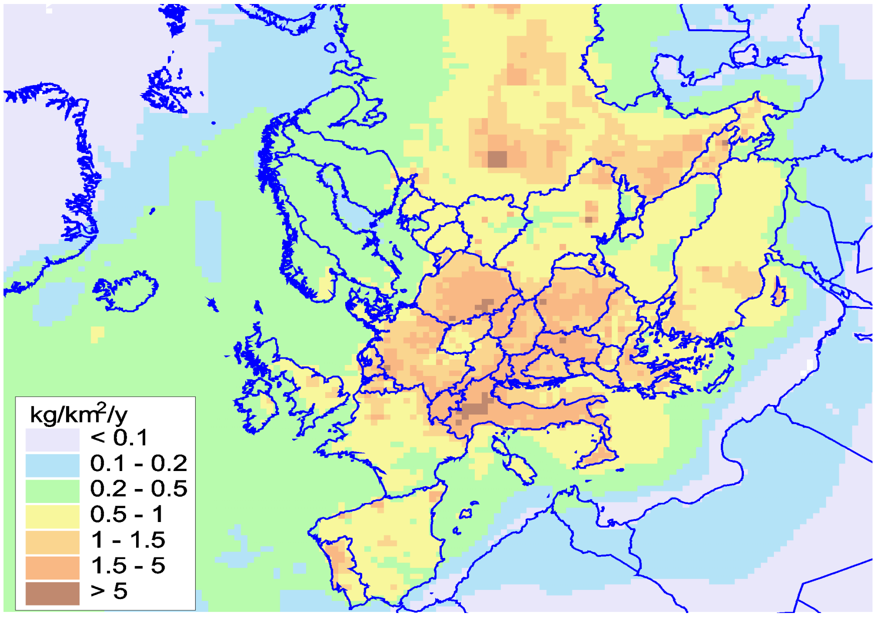


Figure 2.1 Spatial distribution of lead deposition flux in 2001 in European countries (EMEP Report, 2003).

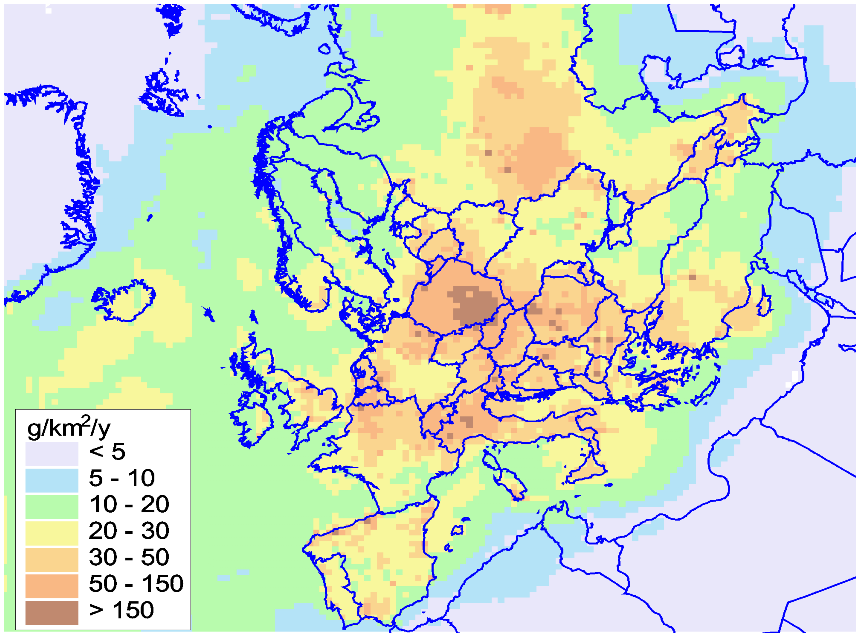


Figure 2.2 Spatial distribution of cadmium deposition flux in 2001 in European countries (EMEP Report, 2003).

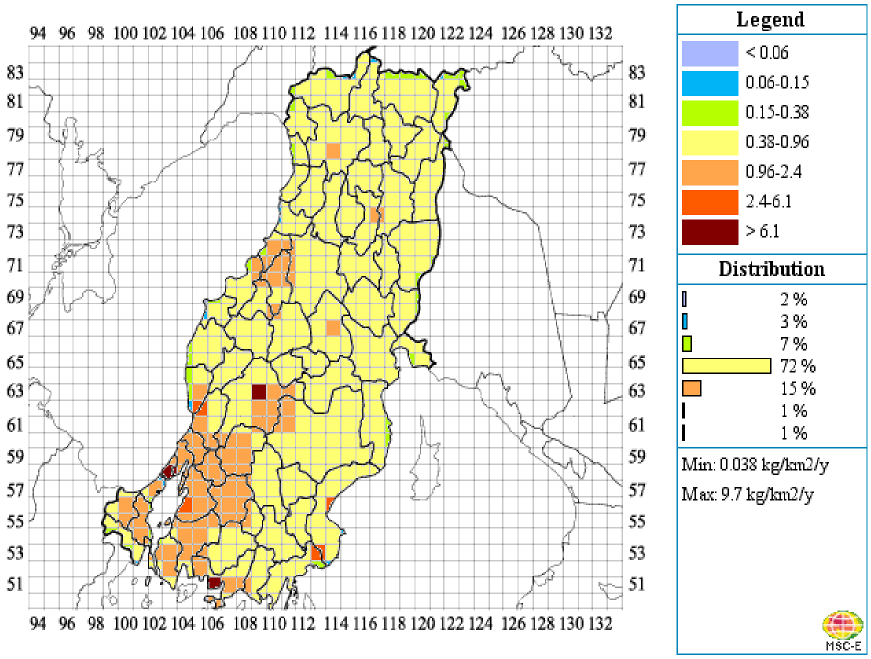


Figure 2.3 Spatial distribution of lead fluxes in 2001 for Turkey, kg km^{-2} yr^{-1} (50x50 km) (EMEP Note, 2003).

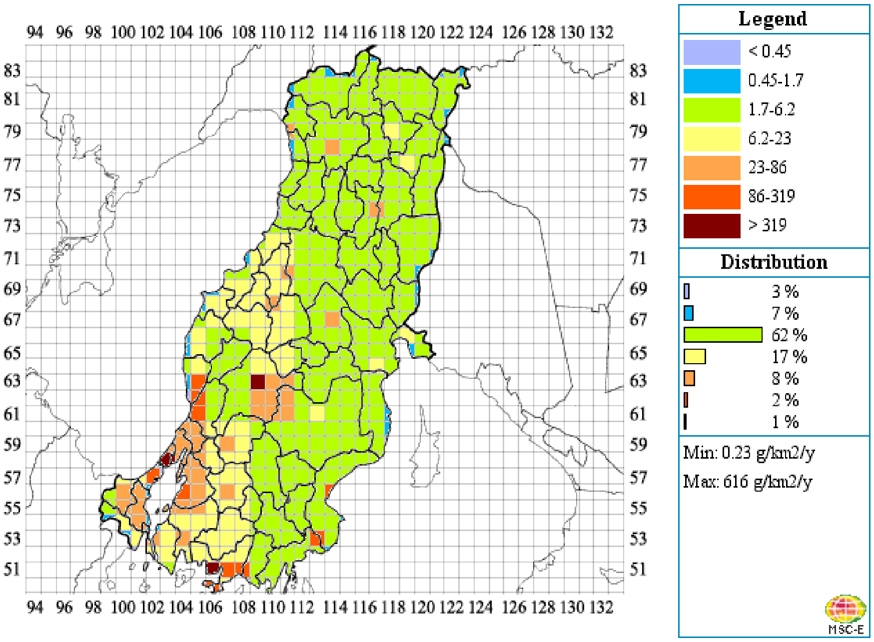


Figure 2.4 Spatial distribution of cadmium fluxes in 2001 for Turkey, g km^{-2} yr^{-1} (50x50 km) (EMEP Note, 2003).

Atmospheric deposition fluxes at urban area were higher than the fluxes of rural area demonstrating the impact of anthropogenic activities in Paris, France by (Azimi et al., 2003).

Long range atmospheric transport of air masses and anthropogenic contaminants in and out of the Russian Arctic was studied by analyzing the 5 day backward and forward air transport trajectories for each day in April and July over a 10 year period from 1986 to 1995 by Vinogradova (2000). It was shown that pollution source contributions vary not only proportionally to their emissions, but also depend on the efficiency of transport of atmospheric pollution to an observation point.

Injuk, Grieken, & De Leeuw (1998) have used the Slinn and Slinn model to calculate dry deposition fluxes from the measured aerosol concentrations for the southern and central North Sea area of Blankenberge, Belgium. Wet deposition fluxes were derived from the mean aerosol concentrations, rainfall instensity and theoretical scavenging rates. Total fluxes of Cr, Pb, Cu, Zn, and Ni were determined as 1300, 1970, 690, 3500, and 650 t yr^{-1} respectively.

Table 2.3 shows the total deposition fluxes of heavy metals reported previously.

Table 2.3 Total deposition fluxes of heavy metals reported previously (kg km^{-2} yr^{-1})

Flux						**Method**	**Location**	**Period**	**Reference**
Cr	**Cd**	**Pb**	**Cu**	**Zn**	**Ni**				
0.60	0.30	5.5	6.0	18.0	-	Total atmospheric deposition collector	Paris, France	Nowember 1999 to July 2000	Azimi et al., 2003
6.4	0.07	12.7	18.6	104.0	8.4	Simple cone-shaped samplers	Pearl River Delta, China	July 2001 to April 2002	Wong et al., 2003
-	0.84	50.7	34.1	85.1	0.84	Wet/dry collector	Belgrade, Yugoslavia	April and May 1999	Tasić et al., 2001
2.4	-	3.7	1.3	6.5	1.2	Modeled	North Sea, Belgium	September 1992 to May 1994	Injuk et al., 1998
2.3	-	2.7	1.3	8.1	1.2	Modeled	Blankenberge, Belgium		
-	0.07	3.1	2.2	80.3	1.4	Total deposition sampler	Ligurian Sea, France	October 1992 to September 1993	Migon, Journel, & Nicolas (1997)
2.7	0.41	2.7	3.5	7.8	7.2	Wet/dry collector	Massachusetts Bay, USA	September 1992 to September 1993	Golomb et al., 1997
-	-	2.8	3.4	9.6	<0.30	A modified precipitation sampler	West Sweden	November 1993 to August 1994	Foltescu et al., 1996

2.4.1 Dry Deposition of Heavy Metals

Several research studies have been performed to investigate the dry deposition of air pollutants. Heavy metals are of particular interest as most of them are toxic to human and ecosystems (Fang, Wu, Huang, & Rau, 2004).

In the absence of precipitation atmospheric dry deposition occurs for the removal of aerosols and gases from the atmosphere onto the surfaces (Fang et al., 2004). Dry deposition involves several processes, such as Brownian motion of particles, sedimentation, and impaction (Yun, Yi, & Kim, 2002). Pirrone, Keeler, & Holsen (1995) reported that dry deposition is affected by a number of meteorological parameters (wind speed, atmospheric stability, relative humidity), pollutant characteristics (chemical form, particle size and shape), and receptor surface characteristics (type and surface roughness).

In direct measurement of dry deposition, an artificial surface is usually used that simulates the natural surface onto which dry deposition is occurring. For this direct measurement of dry deposition by using surrogate sampling techniques are becoming increasingly popular (Cakan, 1999; Odabasi, Sofuoglu, Vardar, Tasdemir, & Holsen, 1999; Shahin, Zhu, & Holsen, 1999; Tasdemir, 1997; Yi, Holsen, & Noll, 1997). Previously, solid surfaces such as Teflon plates, various types of filters, polyethylene buckets, and Petri dishes have been used as surrogate surfaces. These studies have shown that the collector geometry and the surface characteristics have a large impact on the amount of material collected. Dry deposition to water surfaces is one of the key mechanisms that determines the direction and magnitude of pollutant movement in aquatic ecosystems (Shahin, Holsen, & Odabasi, 2002).

Recently, an aerodynamically designed water surface sampler (WSS) has been developed and tested to make direct measurements of atmospheric gases and particles (Cakan, 1999; Odabasi et al., 1999; Shahin et al., 1999; Tasdemir, 1997; Yi et al., 1997). Water surface was used as the surrogate surface for direct measurement of dry deposition of heavy metals by Azimi et al. (2003), Golomb et al. (1997), Morselli et al. (1999), and Sakata & Marumoto (2004), too.

Morselli et al. (1999) evaluated the deposited quantities of several toxic heavy metals such as Cd, Cr, Cu, Ni, Pb, V and Zn that are associated with atmospheric particulate matter at ground level with a dry solid surface sampler and a water layer surface sampler from April 1995 to March 1996 in an urban area of Bologna, Italy. They found that the collecting efficiency of an aqueous surface as a collecting medium were as much as two to three times greater for a number of elements in dry deposition compared to a dry sampler.

Rojas, Grieken, & Laane (1993) noted that the size distribution of atmospheric particulate matter plays an important role in the dry deposition. Atmospheric dry deposition is dominated by coarse particles due to their high deposition flux rates (Injuk et al., 1998; Lin, Fang, Holsen, & Noll, 1993). Yun et al. (2002) reported that large particles (D_p>9 μm) are more important than small particles (D_p<9 μm) in particulate dry deposition of heavy metals. Holsen et al. (1993) have reported that the majority of the flux (>98%) was due to particles of larger than 6.5 μm in size. The PM_{10} coarse fraction accounted for the majority of the PM_{10} dry deposition flux over Lake Michigan during the Lake Michigan Urban Air Toxics Study (LMUATS) according to Pirrone et al. (1995). In contrast, Gao et al. (2002) proposed that dry deposition of trace elements associated with $PM_{2.5}$ was a significant portion of the total atmospheric dry deposition to the New York-New Jersey harbor estuary.

Dry deposition fluxes and deposition velocities for trace metals including Hg, Cd, Cu, Mn, Pb, and Zn in the Tokyo metropolitan area were measured using an improved water surface sampler from May to December, 2002 by Sakata & Marumoto (2004). The results based on 1 yr observations showed that dry deposition plays a significant if not dominant role in trace metal deposition in this urban area; contributing fluxes were ranging from 0.46 (Cd) to 3.0 (Zn) times those of concurrent wet deposition fluxes.

Many researchers explained that the total suspended particulate concentrations and dry deposition fluxes of heavy metals in the daytime period are higher than in nighttime period (Fang et al., 2004; Yun et al., 2002). Higher wind speeds and higher ambient concentrations due to higher human activities in daytime than nighttime may be the reasons for this.

Dry deposition samples were collected on surrogate surfaces of dry deposition plates between September 2000 and June 2001 in Izmir, Turkey by Odabasi, Muezzinoglu, & Bozlaker (2002). Zn was the most abundant anthropogenic element and was followed by Cu and Ni in the dry deposited material in this study. No significant seasonal variation was observed for trace element fluxes. In contrast, Wu, Han, Lin, & Ondov (1994) reported that the concentrations of non-crustal elements, V, Se, Cr and Zn are elevated in winter months, whereas elements with substantial crustal residence, Al, Fe and Mn, are elevated during periods in spring and summer in Chesapeake Bay, USA.

The dry deposition fluxes of heavy metals taken from the previous studies are presented in Table 2.4.

Table 2.4 Dry deposition fluxes of heavy metals reported previously (µg m^{-2} day^{-1})

Flux						Method	Location	Period	Reference
Cr	Cd	Pb	Cu	Zn	Ni				
-	0.16	9.3	21.0	150.0	-	Water surface sampler	Komae, Tokyo	May 2002 to December 2002	Sakata & Marumoto, 2004
-	-	211.0	243.0	5052.0	-	Dry deposition plate	Sha-Lu, Taiwan	August 2003 to September 2003	Fang et al., 2004
15.8	23.9	219.0	123.7	1905.7	129.2	Dry deposition plate	Izmir, Turkey	September 2000 and June 2001	Odabasi et al., 2002
-	-	104.0	56.0	185.0	-	Dry deposition plate	Kunpo, Korea	February 2000 to October 2000	Yun et al., 2002
5.7	-	38.0	63.0	120.0	-	Dry deposition plate	Chicago, USA	December 1993 to October 1995	Yi et al., 2001a
-	-	50.0	60.0	110.0	-	Dry deposition plate	Seoul, Korea	March to November 1998	Yi et al., 2001b
0.21	0.02	2.95	0.49	7.7	-	Modeled	Tel-Shikmona, Israel	Between 1994 and 1997	Herut et al., 2001
-	-	8.0	4.0	11.9	-	Wet/dry collector	Belgrade	In 1999	Tasić et al., 2001
67.6	27.3	680.2	250.3	524.9	78.8	DPM jar	Istanbul, Turkey	March 1999 to April 1999	Alp et al., 1999
-	0.09	7.0	4.4	114.0	1.8	Total deposition sampler	Ligurian Sea, France	October 1992 to September 1993	Migon et al., 1997

2.4.2 Wet Deposition of Heavy Metals

Wet deposition results either from the incorporation of atmospheric particles and gases into cloud droplets by nucleation and their subsequent precipitation as rain or snow (rainout), or from below-cloud scavenging of particles and gases by impaction (washout) (Grantz et al., 2003). Washout is the process of scrubbing of gases and particles by falling droplets. Rainout is the result of incorporating into cloud droplets of gases and particles, or indeed, cloud droplets forming on particle condensation nuclei. In wet deposition, aerosols and gases are dissolved or suspended in precipitation as rain, snow, hail, fog and mist (Golomb et al., 1997). Rainfall and snowfall directly determine the magnitude of wet deposition. Wet deposition is largely a function of precipitation amount and ambient pollutant concentrations (Grantz et al., 2003).

According to some researchers wet deposition is the predominant cleansing mechanism to remove the airborne metals from the atmosphere. For example Grömping et al. (1997) referring to previously obtained data in Germany calculated that more than 90% of the total amount of the metals were found in wet deposition and also concluded that heavy metals are more important in precipitation in urban-industrial areas than at rural measurement sites. These researchers also mentioned the impacts of phase out of lead in gasoline on the air chemistry during the last ten years in Germany.

Although the deposition processes clean the atmosphere, their ultimate result is transfer of toxic atmospheric pollutants into the water and soil environments. Thus soluble heavy metal salts in atmospheric precipitation create ecotoxic effects in the receiving water bodies and in soil. Future research involving particular efforts of to understand the impacts of these pollutants on soil microbiology has been mentioned by Morselli (2004). Hydrolysis rates of the heavy metal salts depositing from the atmosphere into the surface waters or soil water depend on the anion and cation balance and pH of these waters as well as the size and chemical nature of the depositing particles.

A high degree of temporal variability over the annual cycle for metal concentrations in aerosol and precipitation samples collected at a coastal site in New castle was noted by Pike & Moran (2001).

Maneux et al. (1999) reported a strong variability of elemental wet deposition fluxes in the Gironde Estuary and the Arcachon Lagoon, French coastal zone on a daily or weekly time scale. Liu, Zhang, Chen, & Ren (2003) have indicated that the concentrations of most heavy metals in rainwater showed a clear seasonal variation with higher levels in winter and lower levels in summer in Qianliyan Island (Yellow Sea) and Shengsi Island (East China Sea).

The elemental fluxes of As, Cd, Cu, Ni, Pb, Se and Zn discharged from anthropogenic sources were given in Chesapeake Bay Atmospheric Deposition Study by Scudlark, Conko, & Church (1994). Hu & Balasubramanian (2003) reported that Cd, Cr, Co, Cu, Ni, Pb, Zn and V in wet depositions in Singapore were mainly derived from anthropogenic sources, too.

Table 2.5 shows the annual wet deposition fluxes of heavy metals reported earlier.

Table 2.5 Wet deposition fluxes of heavy metals reported previously (kg km^{-2} yr^{-1})

Flux						Method	Location	Period	Reference
Cr	Cd	Pb	Cu	Zn	Ni				
-	0.12	2.7	2.7	18.1	-	Wet-only sampler	Komae, Tokyo	April 2002 to March 2003	Sakata & Marumoto, 2004
0.16	0.05	0.44	0.70	4.1	0.24	An automated collector using trace-element sampler	Washington, USA	In 1998	Conko et al., 2004
0.12	0.04	1.0	0.75	7.4	0.22	Bulk precipitation sampler	Denmark	In 2002	Ellermann et al., 2003
4.2	0.78	8.8	14.6	18.7	10.1	Wet-only sampler	Singapore	In 2000	Hu & Balasubramanian, 2003
0.08	0.12	0.78	0.67	8.3	0.42	-	New Castle, USA	August 1996 to July 1997	Pike & Moran, 2001
0.038	0.035	0.04	0.097	0.4	0.08	Wet-only collector	Cheasepeake and Delaware Bays, USA	September 1995 to August 1996	Kim et al., 2000
-	0.001	0.04	0.02	0.07	-	HDPE funnels and bottles	Paradise, New Zealand	1993-1995	Halstead et al., 2000
1.3	-	3.2	0.88	5.0	0.88	Modeled	North Sea, Belgium	September 1992 to May 1994	Injuk et al., 1998
1.5	-	2.4	0.85	6.3	0.90	Modeled	Blankenberge, Belgium		
0.31	1.5	2.2	0.52	3.4	0.28	Anderson acid precipitation sampler	Ankara, Turkey	November 1992 to July 1994	Kaya & Tuncel, 1997
1.5	0.14	0.57	0.50	2.7	0.62	Wet/dry collector	Massachusetts Bay, USA	September 1992 to September 1993	Golomb et al., 1997
-	0.10	1.2	0.95	8.4	0.30	Wet-only sampler	Germany	In 1994	Grömping et al., 1997
0.09	0.05	0.56	0.26	1.3	0.26	Wet-only collector	Chesapeake Bay, USA	June 1990 to July 1991	Scudlark et al., 1994
-	0.07	4.2	0.23	12.7	0.88	Wet-only sampler	Delta area, Netherlands	1980-1986	Nguyen et.al., 1990

2.5 Ecotoxicity and Test Methods

2.5.1 Principles of Ecotoxicity

Atmospheric heavy metal aerosols are settled by wet or dry deposition to accumulate in soils and water bodies. These accumulated metals cause damages to the organisms living in the surface waters and the soils. The dissolved metals in the rain or water bodies are also absorbed by the plants (Kanellopoulou, 2001).

Bioassays are required to determine the ecotoxicological effects of heavy metals in water. These tests include several representative aquatic species such as bacteria, algae, invertebrates and vertebrates (Villaescusa, Martinez, Pilar, Murat, & Hosta, 1996). Bioluminescent *Vibrio fischeri* has been widely used for measuring the acute toxicity of heavy metals in water samples (Choi & Meier, 2001; Ince, Dirilgen, Apikyan, Tezcanlı, & Üstün, 1999; Mowat & Bundy, 2002a; Ren & Frymier, 2003, 2005; Sorvari & Sillanpaa, 1996; Wang, Wong, Leharne, & Fisher, 1998). The most thoroughly studied bioluminescent bacterium used in toxicity testing is *Photobacterium phosphoreum* (also known as *Vibrio fischeri*), a marine bacterial strain. Methods based on bioluminescent bacteria have the advantages such as small test organism size, large number of organisms, and convenient growing conditions, an easily measured signal and a very quick response (usually of the order of minutes) (Ren & Frymier, 2003).

The marine bacterium *Vibrio fischeri* emits light via a reaction involving a bacterial luciferase enzyme coupled to respiration via NADH and a flavin nucleotide (Calow, 1998). Any change in metabolic activity or disruption of cellular structure due to the presence of toxic substances, results in a rapid change in the rate of bioluminescence (Isenberg & Bulich, 1994). Light output was measured in a photometer equipped with a rotary shutter to protect the photomultiplier tube from ambient light as the test cuvette was installed (Calow, 1998). Test sensitivity is partially explained by the small cell size which gives a high surface to volume ratio

(Isenberg & Bulich, 1994). Reduction of light at 5, 15, or 30 minutes is taken as a measure of toxicity (Calow, 1998).

Isenberg & Bulich (1994) reports that *Vibrio fischeri* has many metabolic pathways which function in respiration, oxidative phosphorylation, osmotic stabilization, and transport of chemicals and nutrients into and out of the cell, and which are located within or near the cytoplasmic membrane. The luciferase pathway functions as a shunt for electrons directly to oxygen at the level of reduced flavin mono-nucleotide. It is also located within the cell membrane complex. This, coupled with the lack of membrane aided compartmentalization of internal functions, gives many target sites at or near the cytoplasmic membrane. These factors all contribute to a rapid response of the organisms to a broad spectrum of toxic substances.

Wang et al. (1998) noted that the luminescent bacteria bioassays are especially good and widely used for acute toxicity tests primarily because they are easy to perform and results can be obtained within a short period of time. Ince et al. (1999) reports that the luminescence of *Vibrio fischeri* is directly associated with respiratory activity and therefore it provides a good indicator of metabolic actions and the general cytotoxicity of a compound or a mixture of compounds.

2.5.2 Methods for Ecotoxicity Tests

Toxic materials in the environment are evaluated by using different ecotoxicity tests. These tests can be groupped as acute toxicity tests, sublethal toxicity tests, lethal toxicity tests, reproduction tests and subacute tests (Conneli & Miller, 1984).

Toxicities of substances are expressed as LC_{50}, EC_{50} or IC_{50} values (Conneli & Miller, 1984). The EC_{50} value can be used to present toxicity potential. EC_{50} value is the concentration at which an organism shows 50% of its maximum response to a toxicant. A toxicant with a small EC_{50} value is therefore more toxic than one with a large EC_{50} value (Ren & Frymier, 2003).

Acute toxicity has been defined as the adverse effects occurring with in a short time of administration of single or multiple doses given within 24 hours. Sub-lethal toxicity test can be done to determine the organism's response to a less severe condition than present in acute effect, and usually after a longer exposure time. Reproduction tests can be done to determine effect of chemicals on the survival, life stages, viability of sperm, fertilization and fertility. Low levels of toxicants in water do not produce mortality in a population of organisms but impair or inhibit reproduction, growth or behavior at subacute toxicity (Conneli & Miller, 1984).

Microbiological ecotoxicity screening tests which are used most commonly are as follows:

Root Elongation Test is based on the inhibition of root growth of *Lepidium sativum* (garden cress) by toxic substances. After 8 days from the test the root length of each cress sprout is measured. The eluate is more toxic as the root is become shorter (Bahadir & Wundram, 1998).

Toxic substances diminish the photosynthetic rate of green algae *Chlamydomonas reinhardtii* in Chlamydomonas Test. By measuring the rate of this reduction the toxicity of the tested substances can be determined. After a 4 h incubation period from the test the photosynthetic rate is measured (Bahadir & Wundram, 1998).

Duckweed Leaf Growth Test is based on the inhibition of leaf growth of *Lemna minor* (duckweed). Lemna plants are placed in the beakers following an 8 day cultivation of the plants, counting the leaves daily. The result is given as the mean daily leaf growth coefficient: The more toxic an eluate, the smaller this coefficient (Bahadir & Wundram, 1998).

The microorganisms used in Genotoxicity Test (MUTATOX^{TM}) are dark mutants of *Vibrio fischeri*. Sublethal concentrations of genotoxic substances induce a remutation of the bacteria. This causes a light emission, which can be detected by a photometer. If the light emission caused by the eluate is at least twice as high as in the control assay this eluate is likely genotoxic (Bahadir & Wundram, 1998).

The biochemical oxygen demand (BOD) is a microbial bioassay test, based on the measurement of respiration of microorganisms. The BOD shows the degree of biological degradability of the substances in a test solution but also shows the toxicity if degradable compounds are present. The respiration test according to OFFHAUS (Sapromat) enables the detection of this toxicity with the addition of an easily degradable carbon source. If a tested eluate is toxic, the degradation of the carbon source and, therefore, the oxygen demand is inhibited (Bahadir & Wundram, 1998).

Test with Luminescent bacteria *Vibrio fischeri* (LUMIStox® and Microtox®) are single celled bacteria of marine origin which produce light energy by a chemical reaction. If the bacteria are incubated with toxic substances their light emission diminishes. The EC_{50} value is automatically calculated by a specially developed software system (Bahadir & Wundram, 1998).

2.5.3 LUMIStox® Toxicity Test

The LUMIStox® test is a bioassay test for measuring the toxicity of environmental samples. LUMIStox® test was developed with luminescent bacteria in conformity with DIN 38412 L34 and L341. The inhibitory effect on the luminescent bacteria was determined by a static test. Certain volumes of test substances were combined with suspension of luminescent bacteria in a cell and measured. This bioassay test enables the user to determine the toxicity of aqueous samples or sample extracts with the help of luminescent bacteria (Dr.Lange, 1994).

Light production could be measured using a variety of photometers. Light emission of the bacteria was measured by a photomultiplier tube in the LUMISmini. The calibration of the measurement instrument is made automatically.

The LUMIStox® test was carried out at 15±1 ^{0}C. Test procedure given in the test manual was strictly followed. Blank solutions were run with the samples. A blank (sample with no added toxicant) was used to correct for the time-dependent change in the light production of the bacteria themselves in order to isolate the toxic effects of the sample alone, as well as to account for small effects due to dilution arising from sample transfer, pipette error, and introduction of reagents.

The luminescent bacteria requires only a short period of time to obtain reliable toxicity results which is one of its major advantages compared with other tests such as fish bioassays, which normally require several days. *Vibrio fisheri* exhibits differing sensitivities for certain compounds depending on the duration of exposure (Mowat & Bundy, 2002a). The time dependent effects on observed toxicity may be due to several processes that interact, including hydrolysis, photodecomposition, membrane diffusion, and chemical reaction with the substrate (Kaiser & Palabrica, 1991). The manufacturer's recommends for metals for a 15 minute exposure time (Microbics Corporation, 1995).

It is known that the species of metal cations may be divided between free ions and some chloro-metal complexes (Tatara, Newman, McCloskey, & Williams, 1998) types of which depend on the total concentrations of Cd, pH and ionic medium (Villaescusa et al., 1996). Very low concentrations used in this study ensured that the metal ions do not precipitate as chlorides and thus the ionic strength impacts are minimized.

In the literature EC_{50} values for assessing the toxicity of single or mixed aqueous solutions of heavy metals are given. This database depends on tests mostly carried out with heavy metal concentrations ranging from a few tens to hundreds mg L^{-1} which is typical in wastewaters, sludge, solid wastes or sediment leachates. However, when very dilute solutions such as 10-100 µg L^{-1} that account for the air pollutant depositions into water bodies are used for determining the EC_{50} values of the metals, EC_{50} values determined by using LUMIStox® test in the literature could not be reproduced. Therefore, toxicity evaluations were made according to the waterborne toxicity levels and Microtox® test.

Jennings, Brandes, & Bird (2001) have investigated the inhibitory effects of 81 organic chemicals, after 5 minute contact time, at eight concentrations using reagents from three commercial assay systems (LUMIStox®, ToxAlert10® and Microtox®) and determined that these bioluminescence based toxicity assays produce very similar results.

Isidori, Ferrara, Lavorgna, Nardelli, & Parrella (2003) have assessed toxic and genotoxic compounds in the urban air of Caserta, South Italy using cuttings from the plant Tradescantia #4430. Toxicity and mutagenesis were determined on the bacterium *Vibrio fischeri* using the Microtox® and Mutatox® systems, respectively. Significant toxic and mutagenic effects were observed at effectively concentrated trace contaminants.

Ren & Frymier (2003) report the inhibition coefficients K_i of seven heavy metals by using Shk1 cells. Shk1 is a bioluminescent bacterial strain genetically engineered for the specific purpose of monitoring influent wastewater to wastewater treatment plants for toxicity. They have arranged K_i and EC_{50} values of the seven heavy metals in increasing order and found that the sequences are in good agreement. They have also denoted that under appropriate conditions, predictions of toxicity could be made based on EC_{50} values that contain kinetic information similar to that contain in predictions using K_i.

A mathematical algorithm has been developed to compute the combined toxicity of mixtures of metal contaminants using toxicity data for the individual components in the mixture by Mowat & Bundy (2002a). The Microtox® bioassay was used to experimentally obtain the toxicity data for a suite of metals (Pb, As, Cd, Cu, Hg, Zn and Cr) that could be potentially present in the environment. Statistically significant deviation of the experimental toxicity of mixtures from the calculated EC_{50} value using the algorithm represents a departure from an additive response, and is classified as synergism or antagonism, as appropriate. Results from binary and ternary metal combinations indicate that all three mixture situations additive, synergistic, and antagonistic are possible.

Mowat & Bundy (2002b) have developed a mathematical algorithm to compute the toxicity of multiple component mixtures acting in an additive manner. A statistical approach was devised to determine the presence of potential interactive effects among mixture components. The algorithm was used three kinds of data to obtain an integrative approach to sediment toxicity assessment: Microtox® toxicity data (EC_{50} values), pollutant concentration measurements, and sequential extraction (SEQ) data to investigate metal partitioning. To simplify the analysis of complex mixtures using a prioritization scheme based on intrinsic toxicity and relative abundance, a toxicity index (TI) was employed as an indicator of adverse ecological impact. This approach was explained that substantial implications for both risk assessment and for remediation strategies, making them more efficient by focusing on the priority pollutants identified.

Two-level factorial experiments were employed for understanding and predicting the toxicity of binary and ternary metal mixtures by Ren, Mee, & Frymier (2004). Toxicity of metal mixtures with concentrations between the respective EC_{10} and EC_{80} values was experimentally measured. Models were fit to the experimental data and the resultant models were of high quality. Interactions between mixture components were indicated by the existence of statistically significant interaction terms in the models. Toxicity predictions based on the models were compared with observed toxicity for binary and ternary metal mixtures. The models developed were not assumed additivity between metals, were simple and interpretable, and given satisfactory predictions of the toxicity of metal mixtures in aqueous solutions without requiring knowledge on synergism or antagonism.

Removal of chromium (Cr) from tannery effluents by recovery of metal also reduces the ecotoxic impact. To develop such a process, columns packed with calcium alginate (CA) beads with or without humic acid (HA) have been used as an adsorbent and tannery effluent was passed through it. Concentration of Cr in beads and in different fractions collected after adsorption was measured. Change in total organic carbon content during the process was also noticed. The fractions were also tested for toxicity towards Microtox® assay. Data showed that the CA beads along with HA could be effectively utilized in removal of 54% Cr and also in reducing the toxicity (Pandey, Pandey, Misra, & Srimal, 2003).

Dalzell et al. (2002) have compared the five rapid direct toxicity assessment methods to determine the toxicity of single toxicants, mixed toxicants and real industrial wastes; nitrification inhibition, respirometry, adenosine triphosphate luminescence and enzyme inhibition. The *Vibrio fischeri* toxicity test was used as a surrogate to compare the various microbial bioassays. The IC_{50} was determined for a number of pollutants including single toxicants Cd, Cr, Cu, Zn, 3,5-dichlorophenol, toluene etc., and 16 industrial effluents. Comparisons were made for the sensitivity, cost of implementation, test costs, relevance, and ease of use. The most sensitive bioassays were *Vibrio fischeri* and Nitrification inhibition; however, this depended in the main on the pollutant and mixtures tested. It is recommended that during assessment of wastewater toxicity a suite of tests be used rather than reliance on one particular test.

Sorvary & Sillanpaa (1996) have investigated the toxicity of heavy metal chelates of EDTA and DTPA by *Vibrio fisheri*, compared the results with the toxicity of uncomplexed heavy metal ions and free EDTA and DTPA. Complexation by either EDTA or DTPA resulted in a significant toxicity decrease with most of the studied metals.

Chen, Huang, Wang, Huang, & Huang (1996) have evaluated the toxicity response for four selected heavy metals by Microtox® and specific oxygen uptake rate (SOUR) tests. At the end of the study, both bioassay methods demonstrated very high sensitivity and almost similar responses of microbial toxicity to heavy metals and organic toxicants while bioluminescence toxicity test proved more effective on toxicity results for both types of toxicants.

The toxicity of cadmium compounds against luminescent bacteria was measured using the Microtox® toxicity bioassay and related to the cadmium species by Villaescusa et al. (1996). Since the Microtox® test is carried out in NaCl (2%) and cadmium forms stable chloro complexes, $NaNO_3$ and $NaClO_4$ have been tested successfully as alternatives to sodium chloride to provide the adequate osmotic protection of the bacteria. However, this modification has not been accepted widely.

Toxicity of zinc, copper, cobalt, and chromium ions and their binary interactions were studied at varying test levels by using a battery of two tests, Microtox® and duckweed with *Vibrio fisheri* and *Lemna minor* as test organisms, respectively (Ince et al., 1999). In the majority of the combinations studied by the two bioassays, the interactions were of antagonistic nature. Additive toxicity was the next frequently predicted interaction in both test results, the frequency being much higher in Microtox® responses than in those of duckweed. Synergism was found to be a rare interaction in Microtox® results, but totally unlikely in duckweed within the selected test combinations.

The EC_{50} values for the studied six heavy metals and corresponding heavy metal concentration ranges of their inputs taken from the previous studies are presented in Table 2.6.

Table 2.6 EC_{50} values for the six heavy metals and corresponding heavy metal concentration ranges of their inputs taken from the literature

Metals	**Studied Concentration Ranges (mg L^{-1})**	**EC_{50} Values (mg L^{-1})**	**Reference**
Cr	20-60	39.0 (5 min)	Ren & Frymier, 2003
	10-1000	66.4±8.0	Mowat & Bundy, 2002a
	60-80	35.2	Ince et al.,1999
	50-500	47.6	Codina et al., 1998
Cd	1-4	1.9 (5 min)	Ren & Frymier, 2003
	10-1000	59.3±6.9	Mowat & Bundy, 2002a
	50-500	40.8	Codina et al., 1998
	0.2-10	0.30	Sillanpaa & Oikari, 1996
	Not cited	34.4	Chen et al., 1996
Pb	4-30	3.7 (5 min)	Ren & Frymier, 2003
	10-1000	0.43±0.18	Mowat & Bundy, 2002a
	Not cited	0.37	Sillanpaa & Oikari, 1996
	Not cited	1.8	Chen et al., 1996
Cu	3-15	9.5	Ren & Frymier, 2003
	10-1000	2.7±1.3	Mowat & Bundy, 2002a
	0.5-2	0.30	Ince et al.,1999
	50-500	0.60	Codina et al., 1998
	Not cited	1.0 (5 min)	Sillanpaa & Oikari, 1996
	Not cited	0.53	Chen et al., 1996
Zn	2-5	1.9 (5 min)	Ren & Frymier, 2003
	10-1000	2.6±0.2	Mowat & Bundy, 2002a
	2-8	1.6	Ince et al., 1999
	50-250	14.5	Codina et al., 1998
	Not cited	2.3	Chen et al., 1996
	Not cited	0.26	Sillanpaa & Oikari, 1996
Ni	3-20	96.2	Ren & Frymier, 2003
	50-1000	170.8	Codina et al., 1998
	Not cited	8.0	Isenberg & Bulich, 1994

CHAPTER THREE
MATERIALS AND METHODS

Sampling and analysis techniques for measuring the concentrations of heavy metals in deposition from air to water surface, dry and wet deposition fluxes of heavy metals as well as the toxicity tests, and calculation methods will be submitted in this chapter.

3.1 Sampling

3.1.1 Sampling Location

Sampling was carried out on a platform 3 meters above the ground in the Dokuz Eylul University Kaynaklar Campus, Buca-Izmir, located in a growing forest about 10 km south of the city center (Figure 3.1). An express way exists about 500 m south and no buildings or similar obstructions exist near the platform. There are no specific heavy metal sources in the area, however, there are residential areas located approximately 2 km southwest of the sampling site. There are numerous industrial plants to the north, south and east of the site about 20-45 km and the metropolitan city of Izmir lies at the west and northwest. Nearest industrial facility is a cement work about 10 km to the north and an open road gravel storage site nearly 3 km to the east of the platform.

Dry and warm climate persists in the area between May and November (Mediterranean climate) and major air movements are from northerly directions in this period with the southerlies occurring as the second important wind group in the winter half of the year. Southerly winds bring precipitation from the Mediterranean Sea.

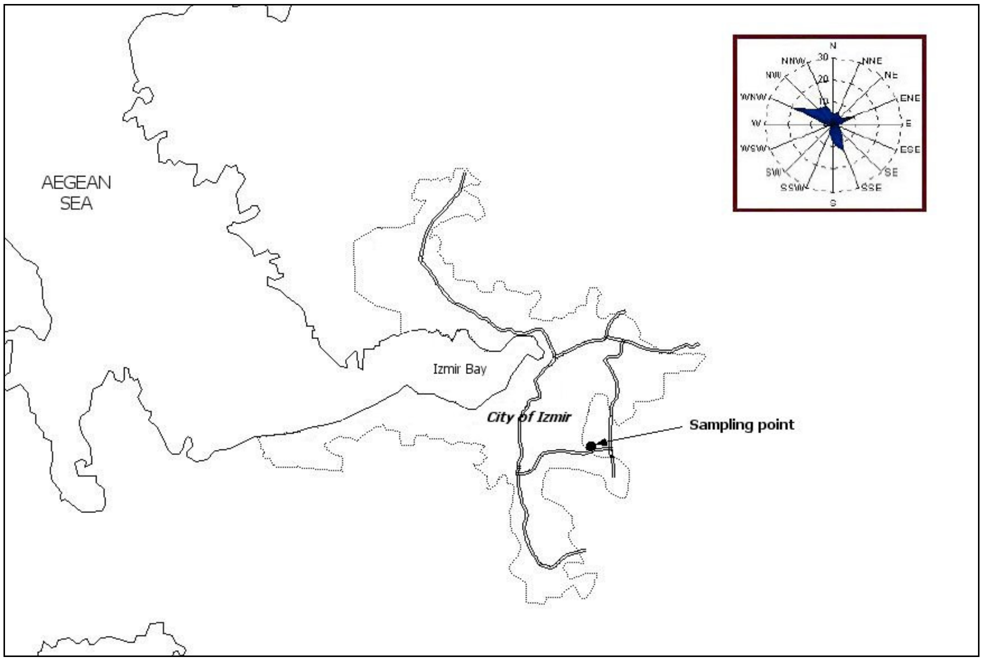


Figure 3.1 Location of the suburban sampling site, and the annual wind rose in the area.

3.1.2 Sampling Program

Dry and wet deposition samples were collected between October 2003 and June 2004 at the study site. Dry deposition samples were collected when there was no rain. Sampling time was 24 h for dry deposition samples. Duration of rainwater sampling varied with the raining period.

Meteorological data was obtained from a meteorological station located on the platform during sampling. The meteorological station has temperature, humidity, pressure, wind direction sensors, a rain gauge, and an anemometer (Davis Instruments, Australia) (Figure 3.2). The data for these parameters by 1 min intervals were stored in a data logger and downloaded to a computer located at the same site. Meteorological parameters in Table 3.1 are the average values over the sampling periods. pH, initial and final volumes of the water samples were measured and recorded both at the beginning and end of the sampling period during dry deposition sampling. Rainwater pH and daily volumes were also measured and recorded. pH and Eh were determined immediately at the end of the sampling duration using WTW Electrode SenTix ORP pH meter calibrated at the neutral pH ranges.

Figure 3.2 View of the sampling platform, sampling equipment and meteorological tower.

3.1.3 Sampling Methods

Heavy metal deposition fluxes were measured by using a WSS in this study. Dry and wet deposition samples were collected in the plate holder with an open surface. This unit has a circular open surface area continuously refreshed with re-circulated water coming up from the center. The water overflows from the carefully designed circular weir all around the plate. In dry deposition sampling the water surface plate was used, but in wet deposition sampling this plate was removed to collect the rainwater in the holder.

Table 3.1 Sampling information and conditions during dry and wet deposition sampling

Sample	***)Date**	****)Sampling Duration (min)**	**Temperature (^{0}C)**		**Wind Speed (m s^{-1})**	******) Wind Direction**	**Rain Intensity, (kg m^{-2} day^{-1})**	*******) Water pH**	**Eh (mV)**
			air	*****)water**					
DD-1	10.06.03-10.07.03	1440	23.6	22.4	2.9	SE-SSE	-	6.48/6.34	262
DD-2	10.22.03-10.23.03	1440	25.2	24.7	5.2	SE-SSE	-	6.21/6.25	258
DD-3	11.04.03-11.05.03	1440	18.6	17.4	3.0	N-NNE	-	5.77/5.74	244
DD-4	11.20.03-11.21.03	1440	13.9	12.5	1.3	ESE	-	6.79/6.78	252
DD-5	12.05.03-12.06.03	1440	7.2	6.7	2.8	N-ESE	-	5.77/5.56	244
DD-6	05.20.04-05.21.04	1440	17.9	17.6	3.0	NNW-WNW	-	7.77/7.68	274
DD-7	05.21.04-05.22.04	1440	19.8	19.5	2.3	NW-WNW	-	6.81/6.81	291
DD-8	05.22.04-05.23.04	1440	19.2	19.1	3.0	ESE-SE	-	6.78/6.77	255
DD-9	06.09.04-06.10.04	1245	23.6	23.3	6.2	N	-	7.77/7.68	268
DD-10	06.10.04-06.11.04	1440	23.6	23.5	6.2	N	-	7.75/7.23	226
DD-11	06.11.04-06.12.04	1440	22.7	22.1	3.6	WNW-NNW	-	6.81/6.81	283
DD-12	06.12.04-06.13.04	1440	24.4	24.1	2.1	ESE-WNW	-	6.75/6.34	238
WD-1	10.08.2003	8	21.2	21.1	7.4	SSE	0.9	7.53	174
WD-2	10.09.2003	31	20.2	19.8	13.8	SSE	5.7	5.94	210
WD-3	10.30.2003	567	8.0	7.4	2.5	SSE	2.2	6.32	261
WD-4	10.31.2003	107	13.7	12.9	2.7	SSE	7.6	7.04	282
WD-5	11.06.2003	359	12.9	11.7	5.1	N	15.6	7.42	284
WD-6	12.31.2003	135	8.7	7.9	0.3	S	7.8	6.54	309
WD-7	01.02.2004	112	8.9	8.5	3.7	SSW	3.9	5.53	381
WD-8	01.05.2004	43	6.5	5.8	3.1	WNW	2.7	6.65	268
WD-9	01.12.2004	327	8.4	8.3	3.1	ENE	1.6	7.62	275
WD-10	01.22.2004	387	7.1	6.7	3.3	NNE	38.3	5.14	216
WD-11	01.30.2004	221	8.1	7.9	4.9	SE	26.4	6.05	315
WD-12	01.312004	101	6.0	5.8	5.1	ESE	5.1	5.06	227
WD-13	06.03.2004	10	20.7	20.3	3.1	S	0.6	7.72	203

*) beginning/end dates of sampling for DD (dry deposition) samples, for WD (wet deposition) samples the date given belongs to the day of the rain sampled.

**) for WD samples total duration of rain on that day

***) measured in the sampler immediately after the collection.

****) direction of the wind of maximum occurrence among all the hourly wind directions for the day.

*****) For DD samples, WSS water pH before/after the sampling, for WD samples, pH of the collected rainwater sample.

The water surface holder has a leading edge to minimize airflow disruptions caused by collector geometry (Figure 3.3). The stainless steel surface plate has a 37.2 cm diameter and 0.65 cm depth and is placed inside the holder at a height which allows the water on the plate to be at the same level with top of the water surface holder. The WSS has a water surface renewal system to maintain a constant water level and control the surface retention time. The pump that provided the water flow through the system was an adjustable liquid pump with wetted parts covered with Teflon. A 4 liter high density polyethylene bottle was used as the water reservoir. Distilled water entered the water surface plate from the center and over-flew from the triangular weirs located at the periphery. The retention time on the water surface was maintained as short as possible (2-4 minutes) in order to prevent losses of deposited heavy metals. All wetted components of WSS and holding tank were washed and rinsed with distilled water prior to each sampling run.

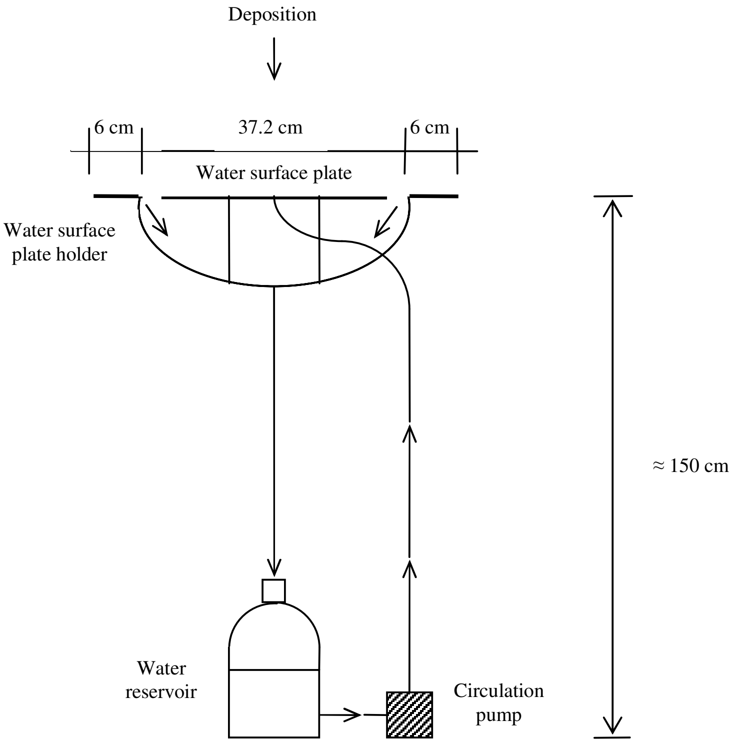


Figure 3.3 Water surface sampler (WSS) that were used to measure heavy metal dry deposition.

To test for the metallic interferences that might come from the stainless steel parts of the WSS, a plastic tank with similar opening size and water depth was placed next to the WSS. Several water blanks were obtained by simultaneous running of the

WSS and the plastic tank with tops in covered position for the sampling duration of about one day.

Rainwater samples were collected in a narrow neck plastic bottle connected to the funnel under the WSS. Sampling was done when the WSS plate was empty. Bottle connection was manually opened after the rain started and closed right after the last rain drops. Heavy metal quantities found for the rainwater samples were corrected for the total volume of the rain on that day.

3.1.4 Sample Handling and Preparation

A schematic representation of the sample handling and pretreatment steps is given in Figure 3.4. At the end of dry deposition sampling, all of the water in the WSS system was transferred into a clean plastic bottle with a plastic stopper and carried to the laboratory where its volume was measured.

The volumes of dry deposition samples were about 0.5-2.3 liters depending on the evaporation height of the day. After thorough shaking a 250 mL aliquot was taken from each sample and immediately filtered through a 0.45 µm Sartorius membrane filter. A hundred milliliter portion of the filtrate was acidified to pH 2 by nitric acid (Merck, 65%) and kept refrigerated in tightly covered plastic bottles until the heavy metals were analyzed for dissolved components. Filters were digested as explained in Section 3.2.1 and analyzed for heavy metals to find the suspended and undissolved fractions of the metals, too. Another 100 mL aliquot of the fresh unfiltered sample was acidified with nitric acid to pH 2 and preserved for total heavy metal analyses. Acidified samples were generally crystal clear with only a few samples which have been filtered before analyses. Filtrates were digested with HNO_3 for analyses of suspended fraction.

Experimental Procedure

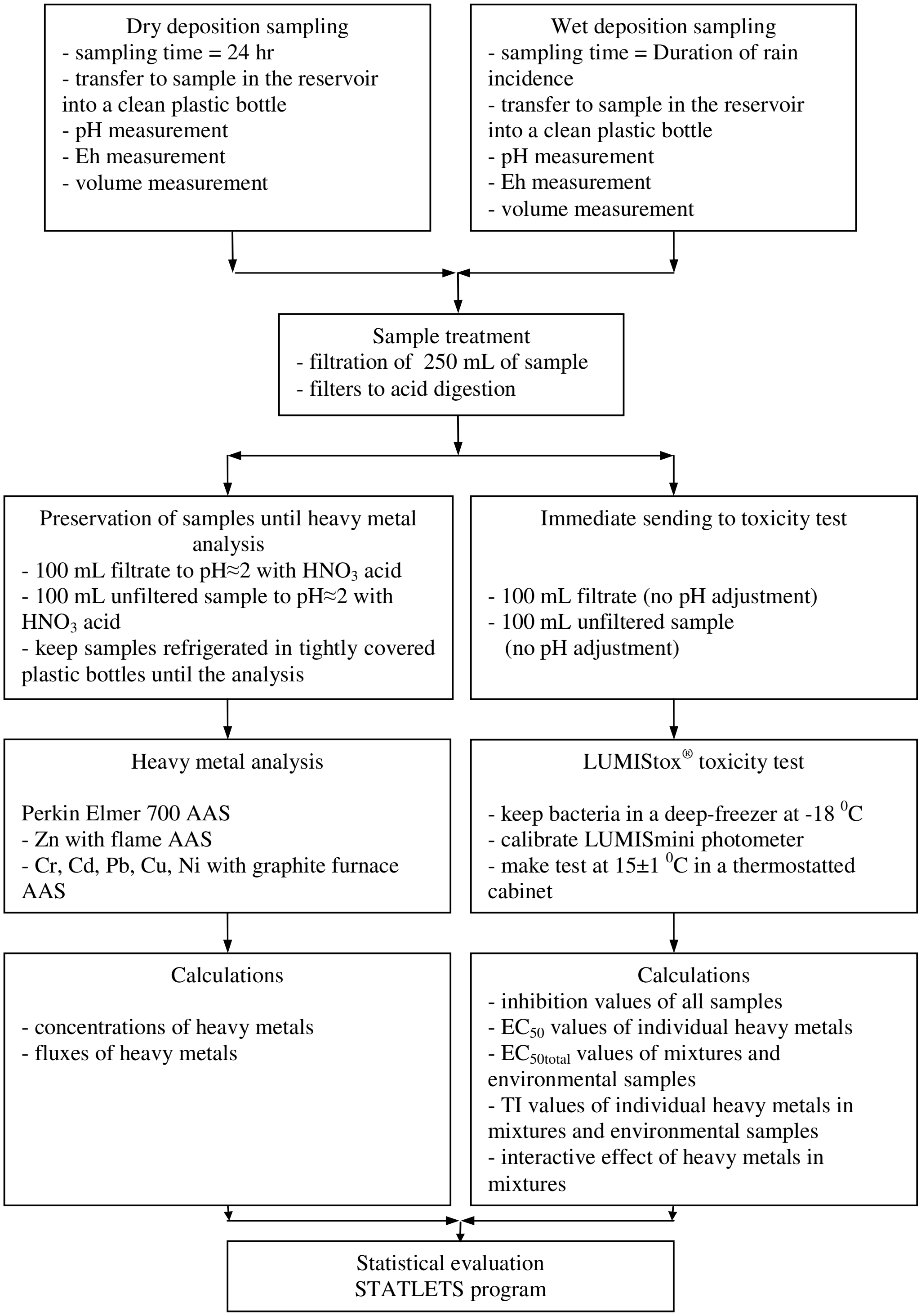


Figure 3.4 Experimental steps from sampling to statistical evaluation.

Fresh unfiltered sample volumes of 100 mL were reserved and used in toxicity testing. Original pH values of the samples were varying between 5.1-7.7 and pH of the initial metal solutions must range between of 5.0 and 9.0 in order to maintain the appropriate conditions for bacterial tests. Therefore, no pH adjustment was needed. Toxicity analyses were made soon after the samples were taken.

3.1.5 Preparation of Synthetic Heavy Metal Solutions

3.1.5.1 Single Synthetic Heavy Metal Solutions

The dry deposition flux values from earlier studies (Bozlaker, 2002; Odabaşı et al, 2002) were used to calculate the expected order of magnitude of the metal concentrations in water samples. In this calculation, the dry deposition metal fluxes were multiplied with the open surface area of the WSS water plate and an average collection water volume of 2 liters in the WSS was assumed. Standard solutions of the metals to represent these expected concentration ranges were prepared from 1 mg L^{-1} stock solutions of reagent grade salts of $Pb(NO_3)_2$, $CuCl_2$, $ZnCl_2$, $NiSO_4.7H_2O$, $CdCl_2.H_2O$, and $K_2Cr_2O_7$ in distilled water. For preparing the stock solutions, salts were dissolved by shaking in water until no visible salt residue remains. The synthetic single and mixed metal solutions used in toxicity testing were prepared by diluting these stock solutions with distilled water to obtain the desired metal concentrations. Different concentrations of one lower, one higher and at least one medium level concentration were used to correspond to the known deposition flux ranges. Analytical standards used in metal analyses were also prepared from these stock solutions, too. Plastic laboratory ware was used for the solutions and test vials.

It was assumed that the metal salts were completely dissolved in water. It is also believed that elements from anthropogenic sources exist mainly as water-soluble forms. The labile metal fraction represents total amounts of soluble elements (Voutsa & Samara, 2002). In this study the soluble fractions of the deposited metals in environmental samples were determined. It was shown that they are highly soluble with average solubility range 75.4-98.8%.

3.1.5.2 Mixture Synthetic Heavy Metal Solutions

Synthetic single and mixed heavy metal solutions were prepared for testing the bacterial behavior in controlled conditions. Single metal solutions consisted of different concentrations of one lower, one higher and at least one medium level concentration of each metal in the expected deposition flux ranges. Six mixed solutions were prepared by keeping one of the metals at 1 mg L^{-1} at a time. The concentrations of the remaining metals were at the minimum concentrations expected due to deposition fluxes of the metals.

3.2 Heavy Metal Analysis

3.2.1 Extraction

After filtration of samples the used membrane filters were placed into clean polyethylene bottles containing 50 mL of 20% nitric acid solution prepared from a stock of acid of high purity (Merck, 65%). The polyethylene bottles were placed into a shaker for 24 hours operating at 60 ^{0}C and 270 rpm to dissolve the metals in the filtered particles in the nitric acid solution. Then the solution was transferred from the bottle into a clean 250 mL Teflon beaker together with 2-3 rinses of distilled water. Teflon beaker was placed on a hot plate at a temperature of about 150-180 ^{0}C to evaporate the acid solution down to a volume of 5-10 mL. An additional 20 mL of nitric acid was added into the beaker, and digestion was continued until 10-15 mL solution was left. After cooling the solution was filtered through another 0.45 µm membrane filter. The filtrates were stored in clean 100 mL polyethylene bottles in a refrigerator until they were analyzed for the undissolved fraction in the environmental samples.

Three unused filter blanks from the same batch were prepared in the same way to determine the contamination from filter extraction procedure. Heavy metal concentrations in suspended form were corrected by subtracting the filter mean blanks from the amounts in the samples.

3.2.2 Analysis

Heavy metals were analyzed by a Perkin-Elmer Model 700 atomic absorption spectrophotometer equipped with a graphite furnace (GFAAS) except Zn. For Zn analysis of samples flame-AAS was used in the same instrument to avoid erratic results in the graphite furnace due to high concentrations in the samples. Background correction was applied using a deuterium lamp with the two-line method. Distilled water, mixtures of distilled water added by nitric acid and procedural blanks such as the covered-top WSS and the plastic tank runs were analyzed for all heavy metals by a GFAAS.

Each injection was 20 µL and injections were made at least in triplicate an auto sampler was used for injections. Process time in the furnace was totally 94 seconds and included five stages for each reading. Furnace temperatures at each stage for metals are given in Table 3.2.

Table 3.2 Temperature and duration program of the GFAAS for each metal

Metal	**Temperature and duration**									
	1.step		**2.step**		**3.step**		**4.step**		**5.step**	
	T (^{0}C)	**Time (sec)**	**T (^{0}C)**	**Time (sec)**	**T (^{0}C)**	**Time (sec)**	**T (^{0}C)**	**Time (sec)**	**T (^{0}C)**	**Time (sec)**
Cr	100	25	140	30	1650	30	2500	5	2600	4
Cd	100	25	140	30	850	30	1650	5	2600	4
Pb	100	25	140	30	700	30	1800	5	2600	4
Cu	100	25	140	30	1000	30	2300	5	2600	4
Zn	100	25	140	30	700	30	1800	5	2600	4
Ni	100	25	140	30	1400	30	2500	5	2600	4

Metal concentrations were determined from calibration curves prepared with known concentrations of the heavy metal standards within expected concentration ranges. Four levels of calibration standard solutions for each heavy metal were prepared by diluting the standard solutions with ultra pure water. These calibration standards were freshly prepared from the stock solutions of 1 mg L^{-1} of the metal salts at high purity reagent grade. For all metals the linear fits of the calibration curves were with $r^2 \geq 0.99$.

3.3 Quality Assurance

Heavy metal analysis of filters and filtrates were aiming at finding the dissolving and undissolving fractions of the deposited material in water. Their sum should give the total deposition of the metal. However, to check the reliability of filtration procedure unfiltered samples were also analyzed to find the total heavy metals after acid digestion. The deviations between summed up filter and filtrate concentrations and corresponding total (unfiltered) concentrations of the samples was not significant at the 99% level.

For quality assurance water blanks were analyzed. To test for the metallic interferences that might come from the stainless steel parts of the WSS, a plastic tank with similar opening size and water depth was placed next to the WSS. Several water blanks were obtained by simultaneous running of the WSS and the plastic tank with tops in covered position for the sampling duration of about one day. The heavy metal concentrations between the closed WSS water blanks, water blanks taken to the site but unused and the plastic tank contents were not different at the 99% level of significance. Thus it was decided that using the WSS with stainless steel parts for sampling the heavy metals in dry and wet deposition samples was acceptable.

All readings have been corrected with the averages obtained from triplicate analyses of at least three blanks obtained from covered top WSS. Limits of quantification (LOQ) were found as the mean blank plus three standard deviations

(Odabasi et al., 1999) in mass units. Mean mass of blank samples and limits of quantifications calculated from these values are presented in Table 3.3.

Table 3.3 Blank mass (μg) and LOQs (μg) of heavy metals (LOQ=Mean blank mass + 3 SD)

Sample		**Mass of Blank Samples for Heavy Metals (μg)**					
		Cr	**Cd**	**Pb**	**Cu**	**Zn**	**Ni**
WSS Blank 1	Dissolved	0.77	0.24	3.69	1.01	8.88	7.44
	Total	0.77	0.25	3.70	1.03	8.89	7.52
WSS Blank 2	Dissolved	0.83	0.27	3.62	0.97	8.95	6.94
	Total	0.84	0.27	3.63	0.97	8.96	6.98
WSS Blank 3	Dissolved	0.82	0.26	3.56	1.02	8.81	7.33
	Total	0.83	0.26	3.57	1.03	8.83	7.35
Mean mass of blanks	Dissolved	0.80±0.03	0.26±0.01	3.62±0.07	1.00±0.03	8.88±0.07	7.24±0.26
	Total	0.81±0.04	0.26±0.01	3.63±0.07	1.01±0.03	8.89±0.07	7.28±0.28
Mean LOQ	Dissolved	0.91	0.29	3.82	1.09	9.08	8.02
	Total	0.92	0.30	3.83	1.10	9.10	8.11
Mean mass of DD samples	Dissolved	9.7±5.7	3.4±1.5	12.3±3.5	9.0±5.5	221.7±77.4	22.2±6.9
	Total	11.3±7.6	4.8±2.2	14.1±4.3	9.8±5.3	240.3±72.5	23.8±7.0
Mean mass of WD samples	Dissolved	13.2±5.5	2.7±1.5	9.5±4.8	16.4±20.9	155.4±238.7	13.1±2.8
	Total	16.4±6.0	3.3±2.1	11.0±6.6	19.4±25.7	189.3±293.2	14.3±3.3

Only the samples with masses of heavy metals exceeding the corresponding mean LOQs were quantified. These results were blank-corrected by subtracting the mean blank amount from the sample amount. Table 3.4 compares the amounts in samples of WSS (dry deposition) and rainwater (wet deposition) to the LOQs.

As can be noted from Table 3.4 the ratios of mass of heavy metals in the samples to the corresponding LOQ varied between 1.7 (Ni in wet deposition samples) and 26.4 (Zn in dry deposition samples). Thus it was assured that all of the results were above the LOQs. In other words, mean LOQ versus metal quantity in samples in percentage is around 5.5-9.4% for Cr, 6.2-10.6% for Cd, 27.0-38.5% for Pb, 5.6-12.2% for Cu, 3.8-5.7% for Zn and 34.5-58.8% for Ni.

Table 3.4 Mass of element in samples (µg)/mean LOQ (µg) ratios of heavy metals

Sample		**Mass of Samples / LOQ**					
		Cr	**Cd**	**Pb**	**Cu**	**Zn**	**Ni**
Dry deposition samples (n=12)	Dissolved	5.0-21.6* (10.7)**	5.0-20.3 (11.6)	1.9-4.9 (3.2)	3.3-21.0 (8.2)	5.6-35.8 (24.4)	1.7-4.1 (2.8)
	Total	5.1-29.5 (12.3)	6.2-26.4 (16.2)	2.0-5.9 (3.7)	4.8-21.0 (8.9)	9.0-36.4 (26.4)	1.8-4.2 (2.9)
Wet deposition samples (n=13)	Dissolved	5.8-24.9 (14.9)	3.4-18.1 (9.4)	1.4-6.2 (2.6)	2.7-60.1 (15.2)	6.3-106.8 (17.6)	1.2-2.5 (1.7)
	Total	6.8-28.8 (18.3)	3.7-28.1 (11.4)	1.5-7.1 (2.9)	2.8-69.3 (17.7)	6.5-129.6 (21.4)	1.3-2.8 (1.8)

* : Range

** : Average

A quality assurance test was also applied during the analysis. In order to verify the AAS readings, calibration curves were tested by using two different methods and efficiencies were calculated as deviations from the known concentrations. At the end of each analysis run three mixed test solutions containing known amounts of the stock solution and added by acidified distilled water similarly prepared as in the acidification step of the samples to pH 2 were analyzed. Results were corrected for the metal concentrations in added acid-water mixture blanks. In this test the heavy metal concentration was chosen so that the test solution at the end is near the third calibration point.

At the second test, solutions were prepared by transferring known volumes of heavy metal stock solution into 500 mL volumetric flasks and adding portions of randomly selected samples. This procedure was repeated using three different samples chosen at random. The difference between the measured heavy metal quantity in the sample and the analysis results of the prepared solutions should correspond to the known heavy metal quantity from the stock solution. Any deviation from the expected mass gave information about the reliability of test results. Measurement results in both of these analytical performance tests described above gave average agreements of 93-97% for Cr, 100% for Cd, 100% for Pb, 93-101.3% for Cu, 92-96% for Zn and 97-114% for Ni.

3.4 Toxicity Tests

Toxicity tests were carried out by using LUMIStox® procedure on both synthetic metal solutions and aqueous samples obtained by collecting the dry and wet deposition in surface waters. Tests were repeated 4-6 times in each case.

LUMIStox® utilizes liquid dried *Photobacterium phosphoreum* (also known as *Vibrio fischeri*) to note the loss of luminescence due to inhibition. The bacterial lots were obtained from Dr.Lange in Germany and transported to laboratory in cold chain. Bacterial batches were kept at in a deep-freezer at -18 ^{0}C until use and were used up within less than six months.

Kaiser & Palabrica (1991) and Villaescusa et al. (1996) denoted that the pH of the test system can vary in the range of $5.0<pH<9.0$ without great effect on the luminescence of the test organisms used. Thus it must be assured that the pH of the initial metal solutions must range between of 5.0 and 9.0 in the test vials in order to maintain the appropriate conditions for bacterial tests. Therefore, pH values of the all solutions and samples were tested before introduction into the test vials. Eh values of original metal solutions were also measured and evaluated together with the pH values to ensure that the metal ions do not precipitate in the chloride-rich medium in the test vials. This is in parallel with the information that any pH adjustment could lead to precipitation of some of the heavy metals thus harming the ecotoxicity test (Ren & Frymier, 2003).

The bioassay test was completely conducted at 15±1 ^{0}C in a thermostatted cabinet. Blank solutions were run parallel with the samples to correct time-dependent changes in the light production of the bacteria.

In this study toxicity was measured as percent inhibition of light emission (corrected for the light loss in the control) before and after 5, 15, and 30 minutes incubation periods of the reconstituted bacteria in synthetic solutions and environmental samples. Based on first test results 5 and 30 minutes exposure readings were ruled out due to their lack of reproducibility and an optimum exposure time of 15 minute was selected for use. Literature studies (Ince et al., 1999; Mowat & Bundy, 2002a) and the manufacturer's recommendations also confirm to select 15 minutes exposure time.

Samples were adjusted to 2% salinity by using of 0.3 g NaCl per 15 mL of the solution in order to optimize the environmental conditions for the bacteria of marine origin. One vial of reactivation solution was thawed at room temperature in a water bath for 2 minutes just before reactivation and shaken vigorously. The liquid dried bacteria were reactivated by adding 0.5 mL reactivating solution, the tube was allowed for 15 minute without shaking. The suspended luminescent bacteria were mixed with the remaining reactivation solution. From this bacterial suspension 0.5 mL was transferred into the glass cuvette and allowed for 15 minute before use.

Light emission of the bacteria was measured using the special photometer LUMISmini which is automatically calibrated (Figure 3.5). The measuring well of the instrument was opened and the first measuring cuvette was placed. The lid was closed again. Arrow key was followed. Initial luminescence of each test batch was measured following a certain time interval. Measuring cuvette was removed and 0.5 mL of corresponding sample was added immediately. Same procedure was repeated with other measuring cuvettes after 5, 15, 30 minutes incubation periods.

Bacterial responses in each new bacteria package were checked to confirm the dichromate reference point provided in the test kit. An average percent inhibition of 45% for the reference solution of 4 mg L^{-1} $K_2Cr_2O_7$ was found that checks well with the reference value of 42% given by the manufacturer for the bacterial lot used.

Before the environmental samples were analyzed for bacterial toxicity, synthetic single metal and mixed metal solutions simulating the environmental mixtures were prepared and toxicities were determined. This was to see the impacts of individual heavy metal components and their mixtures on bacterial die-off.

Figure 3.5 The special photometer LUMISmini used during the toxicity tests in this study.

3.5 Calculations

3.5.1 Calculation of Heavy Metal Concentrations

Absorbance values obtained from GFAAS were plotted against the chemical concentrations in solutions and samples. The calibration curve prepared from known standards was used to find the concentrations of heavy metals. Corrections and calculations were made by using a model developed in Microsoft Excel Program.

3.5.2 Calculation of Dry and Wet Deposition of Heavy Metals

Dry deposition fluxes were calculated by dividing the amount of trace metal deposited by the area of the WSS plate and the sampling time. Equation 3.1 was used to estimate the dry deposition fluxes:

$$F = \frac{M_d}{A x T} \tag{3.1}$$

where F is the trace metal deposition flux (µg m^{-2} day^{-1}), M_d is the collected mass of the metal in the day of the sampling (µg), A is the deposition sampler collection area (m^2), and T is the sampling duration on sampling day (day).

Wet deposition fluxes were calculated by multiplying heavy metal concentrations in rainwater samples and the amount of precipitation recorded for each collection period. This calculation method was used by Deboudt et al. (2004), Halstead et al. (2000), Hu & Balasubramanian (2003), Kaya & Tuncel (1997), Luo (2001), Migon et al. (1997), Özsoy & Örnektekin (2005), and Sakata et al. (2006). Equation 3.2 was used to estimate the wet deposition fluxes:

$$F_w = C_w x V_w \tag{3.2}$$

where F_w is the calculated metal flux from wet deposition (µg m^{-2} day^{-1}), C_w is the metal concentration measured in precipitation (µg L^{-1}), and V_w is the total precipitation height during the sampling day (mm day^{-1}).

Estimations of annual dry and wet deposition rates were found using the following methods:

a- As there were only 31 rainy days during the study year, the working atmospheric cleansing mechanism was dry deposition for the remaining dry weather days of 334 days of the year. Thus annual dry deposited heavy metals were estimated by taking the average daily dry deposition rate in (µg m^{-2} day^{-1}) based on 12 days of dry deposition measurement data and multiplying it with the total number of dry deposition days which was 334 for the study year.

b- Wet annual deposition fluxes (µg m^{-2} yr^{-1}) was calculated by multiplying the daily wet deposition fluxes (µg m^{-2} day^{-1}) and the ratios between the annual precipitation height (406.8 mm yr^{-1}) and the daily precipitation height during the sampling days. And then annual flux was calculated by averaging these values.

3.5.3 Calculation of EC_{50} Values of Single Heavy Metals

The percentage inhibition effect of metals on the light emission was calculated by using the procedure recommended by Dr.Lange (1994).

“fK” which is the temporary correction factor determined from the control measurements was calculated by using the Equation 3.3:

$$fK = \frac{I_{tc}}{I_{oc}} \tag{3.3}$$

where I_{tc} is the light emitted by the control solution at times (5, 15, 30 minutes), and I_{oc} is the light emitted by the control solution at zero time.

The corrected intensity of luminescence (I_{ts}) was calculated from the I_{os} values of the test solutions. I_{ts} was calculated by using Equation 3.4:

$$I_{ts} = fK \times I_{os} \tag{3.4}$$

where I_{os} is the light emitted by the test solution at time zero.

The percentage inhibition (% Inhibition) of a test substance on the light emission was calculated after exposure times of 5, 15 and 30 minutes:

$$\% \text{ inhibition} = \left[\frac{(I_{ts} - I_{os})}{I_{ts}} \right] \times 100 \tag{3.5}$$

For synthetic single heavy metal solutions EC_{50} values were determined according to the methods proposed by Mowat & Bundy (2002a) and Jennings et al. (2001). One of these methods Jennings et al. (2001) suggest to gamma values were plotted against their corresponding chemical concentrations, after first transforming all data to natural logs (Ln), to generate (Ln gamma/Ln concentration) curves for each compound in each test system. The data for percentage inhibition obtained in each experiment were converted to gamma values:

$$\text{gamma} = \left[\frac{\%\text{inhibition}}{(100 - \%\text{inhibition})}\right] \tag{3.6}$$

Values falling within the 10-90% inhibition range were used to fit a straight line to the Ln-transformed data by linear regression and the resulting equations used to calculate the EC_{50} values. The EC_{50} has been calculated from linear regression equations of dose-response curves of the form:

$$\text{Ln } y = m \text{ Ln } x + c \tag{3.7}$$

where Ln is the natural logarithm, y the value for gamma, x is the dose and m and c are the slope and intercept, respectively. The individual values for the slope and intercept for each compound may be used to calculate any desired gamma value and thus any required effective concentration.

For synthetic single heavy metal solutions EC_{50} values were determined also according to another method proposed by Mowat & Bundy (2002a). To determine the EC_{50} value, inhibitions versus the concentrations have been plotted for single metal standards. The concentration, gamma value and slope (m_i) of this curve for any metal (i) are interrelated by,

$$\log C_i = m_i \log \Gamma_i + \log EC_{50_i} \tag{3.8}$$

$$\Gamma_i = \frac{I_{oi} - I_{ti}}{I_{ti}} \tag{3.9}$$

where C_i is the concentration of toxicant (i) in the mixture, Γ_i is a measure of the light attenuation; I_{oi} is the light intensity at the beginning, and I_{ti} is the remaining light intensity after time (t). At the point where $\Gamma_i=1$ the light extinction is 50% which corresponds to the concentration of the toxicant which is EC_{50} by definition.

3.5.4 Calculation of $EC_{50total}$ Values of Synthetic Metal Mixtures and Samples

In order to include interactive effects in multi-element mixtures, Mowat & Bundy (2002b) formulated a mathematical model to calculate the combined Γ_{total} of a mixture. To compute the $EC_{50total}$ value of a metal mixture, a mathematical algorithm proposed by Mowat & Bundy (2002b) was followed. This model is expanded to a six component formula for the number of metals in this study to obtain the overall toxicity, $EC_{50total}$. This overall toxicity indicator for the mixed solutions was calculated using EC_{50} of each metal in a mixture, its slope m_i, and dissolved concentrations of heavy metals in samples.

In order to include interactive effects, Mowat & Bundy (2002a) formulated an expression for the combined Γ_{tot} of a mixture. According to this when six heavy metals investigated in this study are contributing to the overall toxicity, the cited algorithm was transformed into a six-component model;

$$\Gamma_{tot}=\sum_{i=1}^{6}\Gamma_i+\frac{1}{2!}\sum_{i,j=1_{i\neq j}}^{6}\Gamma_i\Gamma_j+\frac{1}{3!}\sum_{i,j,k=1_{i\neq j\neq k}}^{6}\Gamma_i\Gamma_j\Gamma_k+\frac{1}{4!}\sum_{i,j,k,l=1_{i\neq j\neq k\neq l}}^{6}\Gamma_i\Gamma_j\Gamma_k\Gamma_l+\frac{1}{5!}\sum_{i,j,k,l,m=1_{i\neq j\neq k\neq l\neq m}}^{6}\Gamma_i\Gamma_j\Gamma_k\Gamma_l\Gamma_m+\frac{1}{6!}\sum_{i,j,k,l,m,n=1_{i\neq j\neq k\neq l\neq m\neq n}}^{6}\Gamma_i\Gamma_j\Gamma_k\Gamma_l\Gamma_m\Gamma_n \quad (3.10)$$

where indices i of 1 to 6 refer to the six metals, and gamma at the left hand side is the total light attenuation. Terms at the right hand side expresses contributions of individual components on the overall toxicity. Inhibition data obtained for synthetic mixtures and the environmental samples created by daily dry and wet deposition fluxes were used in computing the Γ_{total}. This accounts for the toxic contribution of each individual chemical ($\Gamma_1+\Gamma_2+\Gamma_3+\Gamma_4+\Gamma_5+\Gamma_6$) as well as any interaction between components in the mixture. Use of equations 3.8, 3.9 and 3.10 implicitly assumes that all components in the mixtures are equally bioavailable Mowat & Bundy (2002a).

If equations of the form of equation 3.8 are solved for Γ_i of each component and the proportionally constants α, β, a, b, c (which represents the ratios of C_1 to C_2, C_1 to

C_3, C_1 to C_4, C_1 to C_5, and C_1 to C_6, respectively) are introduced, the Γ_i values can be written as:

$$\Gamma_1 = \left[\frac{C_1}{EC_{50_1}} \right]^{\frac{1}{m_1}} \tag{3.11}$$

$$\Gamma_2 = \left[\frac{C_1}{\alpha \times EC_{50_2}} \right]^{\frac{1}{m_2}} \tag{3.12}$$

$$\Gamma_3 = \left[\frac{C_1}{\beta \times EC_{50_3}} \right]^{\frac{1}{m_3}} \tag{3.13}$$

$$\Gamma_4 = \left[\frac{C_1}{a \times EC_{50_4}} \right]^{\frac{1}{m_4}} \tag{3.14}$$

$$\Gamma_5 = \left[\frac{C_1}{b \times EC_{50_5}} \right]^{\frac{1}{m_5}} \tag{3.15}$$

$$\Gamma_6 = \left[\frac{C_1}{c \times EC_{50_6}} \right]^{\frac{1}{m_6}} \tag{3.16}$$

When equations 3.11-3.16 are inserted into equation 3.10, mixture Γ_{total} could be described as a function of C_1 which is the concentration of an arbitrarily selected component (1) and can be denoted as $\Gamma_{total(C1)}$. By definition at the $EC_{50total}$ for six component mixture, Γ_{total} will be equal to one. When $\Gamma_{total(C1)}$ is equal to one, C'_1 can be solved. Since C'_1 is calculated and the proportion of each component in the mixture is known, $EC_{50total}$ for the mixture can be calculated as:

$$EC_{50_{total}} = C_{total} = \frac{C'_1}{P_1} \tag{3.17}$$

where P_1 is the proportion of component 1 (chosen arbitrarily from among the pollutant constituents) in the mixture.

Computations were made using a model which preparing of Microsoft Excel Program for multiple component mixtures.

3.5.5 Calculation of Toxicity Index Values of Synthetic Metal Mixtures and Samples

EC_{50} results were also converted into "Toxicity Index" to indicate the contribution of particular metals in the mixed solutions and samples. Toxicity Index (TI) is the ratio of dissolved metal concentration to EC_{50} of the metal and is used as a measure of the potential hazards posed by a particular metal to simplify the determination of the overall $EC_{50\text{total}}$ of complex mixtures. TI is defined such that

$$\mathrm{TI} = \frac{\text{concentration of particular metal}}{\mathrm{EC_{50}}\text{ for the particular metal}} \tag{3.18}$$

Large TI values indicate a high potential contribution to overall toxicity (Mowat & Bundy, 2002b).

3.5.6 Calculation of Interactive Toxicity Effects of Synthetic Metal Mixtures

Aquatic organisms in natural water systems are generally exposed to mixtures of metals, which may multiply, suppress, or add the effects of single components (Ince et al., 1999). Interactive metal effects are additive, synergistic, or antagonistic. Additive effect occurs when the combined effect of two or more metals is equal to the sum of the effect of each metal given alone. Synergism refers to the effect caused when exposure to two or more chemicals at as time results in health effects that are greater than the sum of the effects of the individual chemicals. Antagonism is the opposite of synergism. It is the situation where the combined effect of two or more compounds is less toxic than the individual effects (Canada's National Occupational Health & Safety Resource [CCOHS], 2004).

A method was developed to predict mixture toxicity in different concentrations ratios. This method Equation 3.19 can be used to determine the EC_{50M} value for a mixture of multiple toxicants (Mowat, 2000):

$$EC_{50M} = \frac{C_M}{\frac{C_1}{EC_{50_1}} + \frac{C_2}{EC_{50_2}} + \ldots + \frac{C_N}{EC_{50_N}}} \qquad (3.19)$$

where EC_{50M} is the EC_{50} value for the mixture and C is the molar concentration of the mixture (C_M) or of the individual mixture components (C_1, C_2, …,C_N). C_M is the total of $C_1 + C_2 + \ldots + C_N$.

When six heavy metals investigated in this study are contributing to the overall toxicity, the equation 3.19 was transformed into a six-component model;

$$EC_{50M} = \frac{C_1 + C_2 + C_3 + C_4 + C_5 + C_6}{\frac{C_1}{EC_{50_1}} + \frac{C_2}{EC_{50_2}} + \frac{C_3}{EC_{50_3}} + \frac{C_4}{EC_{50_4}} + \frac{C_5}{EC_{50_5}} + \frac{C_6}{EC_{50_6}}} \qquad (3.20)$$

Discrepancies between calculated and observed values were attributed to small differences in the mechanism of toxic action of the compounds and to interactions between the chemicals in the mixtures. When the calculated EC_{50} exceed the observed EC_{50}, the interaction was classified as synergistic. Antagonism occurred if the opposite were true (Mowat, 2000).

3.6 Statistical Evaluation

Statistical evaluations were made to study the relationships between two or more sets of data among heavy metal concentrations and fluxes, toxicity values, and meteorological parameters by using STATLETS statistical program. Basic statistics and multiple regression analysis were applied between variables. The p value and correlation coefficients obtained from statistical analysis were used to determine the significance of the relations at the 95% confidence level. The correlation coefficients range between -1 and +1 and measure the strength of the linear relationship between the variables. Also displayed is a p value which tests the statistical significance of the estimated correlations. The p values below 0.05 indicate statistically significant non-zero correlations at the 95% confidence level. In addition paired t-test was used to determine whether there is no significant difference among some data sets. When t_{stat} value is small than t_{critic} value obtained from paired t-test, there is no significant difference among two data sets.

CHAPTER FOUR

RESULTS AND DISCUSSION

Heavy metal concentrations in wet deposition (rainwater) samples; dry and wet deposition fluxes; EC_{50} values of synthetic single heavy metal solutions; percent inhibitions, $EC_{50total}$ and toxicity index (TI) values, and interactive toxicity effects in all samples and synthetic metal mixed solutions based on the bacterial bioassay tests will be submitted and discussed in this chapter.

4.1 Heavy Metal Concentrations in Blank and Wet Deposition Samples

Volume weighted concentrations of heavy metals in blank and rainwater samples were reported as dissolved, suspended and total concentrations. Total concentrations were calculated as the sum of the dissolved and suspended forms. To defend this calculation procedure separate tests for the total (unfiltered) samples were analyzed and the measured and calculated concentrations were statistically analyzed to show the agreement between the two data sets. Deviations were insignificant, therefore summed up values were used in the evaluations.

4.1.1 Heavy Metal Concentrations in Blank Samples

Three from each of the field (WSS) blanks, unused distilled water blanks and unused distilled water+nitric acid blanks were collected and analyzed during the study period. Heavy metal concentrations in WSS blanks are given in Table 4.1. Another set of blanks consisting of distilled water used in the WSS and experimental studies are analyzed and the results are shown in Table 4.2. Also to judge for the contamination from added acids heavy metal analyses were carried out on the mixtures of distilled water+nitric acid prepared in the same way as used in the study. Table 4.3 shows the heavy metal concentrations in blanks of distilled water added by nitric acid.

Table 4.1 Heavy metal concentrations of WSS blank samples

Sample		**Heavy Metal Concentrations (μg L^{-1})**					
		Cr	**Cd**	**Pb**	**Cu**	**Zn**	**Ni**
WSS blank sample 1	Dissolved	0.5	0.2	1.9	0.5	4.8	3.9
	Suspended	0.0	0.0	0.0	0.0	0.0	nd
	Total	0.5	0.2	1.9	0.5	4.8	3.9
WSS blank sample 2	Dissolved	0.5	0.2	1.9	0.6	4.8	4.2
	Suspended	0.0	0.0	0.0	0.0	0.0	0.0
	Total	0.5	0.2	1.9	0.6	4.8	4.2
WSS blank sample 3	Dissolved	0.5	0.2	1.9	0.6	4.9	4.0
	Suspended	0.0	0.0	0.0	0.0	0.0	0.0
	Total	0.5	0.2	1.9	0.6	4.9	4.0
Average concentration ±SD*	Dissolved	0.5±0.0	0.2±0.0	1.9±0.0	0.6±0.1	4.8±0.1	4.0±0.2
	Suspended	0.0	0.0	0.0	0.0	0.0	0.0
	Total	0.5±0.0	0.2±0.0	1.9±0.0	0.6±0.1	4.8±0.1	4.0±0.2

*: SD : Standard deviation.

Table 4.2 Heavy metal concentrations of distilled water

Sample	**Heavy Metal Concentrations (μg L^{-1})**					
	Cr	**Cd**	**Pb**	**Cu**	**Zn**	**Ni**
DW 1*	0.4	0.1	1.6	0.2	2.2	3.3
DW 2*	0.4	0.1	1.4	0.2	2.1	3.1
DW 3*	0.4	0.1	1.6	0.2	2.1	3.3
Avg.conc.±SD	0.4±0.0	0.1±0.0	1.5±0.1	0.2±0.0	2.1±0.1	3.2±0.1

*: DW 1, DW 2 and DW 3 were defined to distilled water samples were used in this study.

Table 4.3 Heavy metal concentrations of distilled water added by 0.1 mL nitric acid per 100 mL water down to pH 2

Sample	**Heavy Metal Concentrations (μg L^{-1})**					
	Cr	**Cd**	**Pb**	**Cu**	**Zn**	**Ni**
Sample 1*	0.5	0.1	1.9	0.5	2.1	3.6
Sample 2*	0.5	0.1	1.8	0.5	2.2	3.7
Sample 3*	0.4	0.1	1.9	0.5	2.2	3.5
Avg.conc.±SD	0.5±0.1	0.1±0.0	1.9±0.1	0.5±0.0	2.2±0.1	3.6±0.1

*: Sample 1, Sample 2 and Sample 3 were defined to distilled water added nitric acid mixtures were used in this study.

As shown in Table 4.1-4.3, Zn and Ni had increased concentrations in the procedural blanks compared to the unused and acidified distilled water blanks for the WSS and sample pretreatment. Analysis showed that about 55% of the Zn concentrations and 78% of the Ni concentrations in procedural blanks came from distilled water and the rest were due to other factors possibly including the contamination due to steel parts of the WSS.

Concentration of Cd in the procedural blank was higher than 0.1 μg L^{-1}. In the distilled water blanks added by nitric acid no extra Cd appeared but all other metals have increased compared to the distilled water. For example, additional amounts of 20% of Cr and Pb were due to acid addition to the distilled water blanks.

4.1.2 Heavy Metal Concentrations of Rainwater (Wet Deposition) Samples

The volume weighted average concentrations for the six elements observed in the rainwater (wet deposition) samples are summarized in Table 4.4. For these samples the average total heavy metal concentrations for Cr, Cd, Pb, Cu, Zn and Ni were found as 17.1±8.5, 3.1±1.6, 6.6±4.1, 19.5±24.8, 184.2±224.0, and 6.7±2.6 in μg L^{-1}, respectively.

A statistically significant relationship between two heavy metals indicates that the source and the characteristics for both heavy metals may be the same, although the metal concentrations themselves might be showing considerable variability. Any variability may be due to changing meteorological conditions affecting the transport time of the pollutants, chemical speciation and strengths of pollution emissions sources contributing the pollution of the air.

Table 4.4 Heavy metal concentrations in rainwater samples

Sample		**Heavy Metal Concentrations (μg L^{-1})**					
		Cr	**Cd**	**Pb**	**Cu**	**Zn**	**Ni**
WD-1	Dissolved	25.5	3.1	9.0	34.7	187.2	5.3
	Suspended	10.8	0.3	4.1	20.1	313.4	8.9
	Total	36.3	3.4	13.1	54.8	500.6	14.2
WD-2	Dissolved	6.6	2.5	8.2	39.0	624.5	8.4
	Suspended	5.1	0.2	6.8	10.3	130.7	nd
	Total	11.7	2.7	15.0	49.3	755.2	8.4
WD-3	Dissolved	13.1	0.7	1.8	2.2	68.7	4.2
	Suspended	nd	nd	nd	0.2	8.6	nd
	Total	13.1	0.7	1.8	2.4	77.3	4.2
WD-4	Dissolved	5.8	0.6	4.8	5.2	52.9	5.5
	Suspended	0.7	nd	nd	nd	5.3	nd
	Total	6.5	0.6	4.8	5.2	58.2	5.5
WD-5	Dissolved	4.8	2.7	12.1	9.9	84.9	6.3
	Suspended	0.6	2.2	nd	nd	4.3	nd
	Total	5.4	4.9	12.1	9.9	89.2	6.3
WD-6	Dissolved	17.7	4.5	4.7	8.3	83.6	6.0
	Suspended	6.1	nd	nd	nd	nd	nd
	Total	23.8	4.5	4.7	8.3	83.6	6.0
WD-7	Dissolved	24.4	3.4	4.3	2.2	73.5	5.3
	Suspended	4.1	1.4	nd	nd	10.8	nd
	Total	28.5	4.8	4.3	2.2	84.3	5.3
WD-8	Dissolved	15.9	2.3	4.0	2.8	58.6	5.8
	Suspended	1.7	0.9	nd	nd	3.3	nd
	Total	17.6	3.2	4.0	2.8	61.9	5.8
WD-9	Dissolved	18.0	2.1	4.2	2.7	65.7	5.6
	Suspended	0.4	1.1	nd	nd	6.4	nd
	Total	18.4	3.2	4.2	2.7	72.1	5.6
WD-10	Dissolved	12.3	5.2	7.4	67.0	72.1	5.1
	Suspended	0.7	nd	nd	10.0	nd	nd
	Total	13.0	5.2	7.4	77.0	72.1	5.1
WD-11	Dissolved	10.6	0.8	3.6	2.5	59.2	4.7
	Suspended	6.9	nd	nd	nd	6.6	nd
	Total	17.5	0.8	3.6	2.5	65.8	4.7
WD-12	Dissolved	6.3	3.0	5.2	13.1	64.7	6.7
	Suspended	6.2	0.2	nd	0.2	nd	nd
	Total	12.5	3.2	5.2	13.3	64.7	6.7
WD-13	Dissolved	14.4	2.4	5.3	20.3	379.6	8.8
	Suspended	3.1	0.2	1.3	3.4	30.3	nd
	Total	17.5	2.6	6.6	23.7	409.9	8.8
Avg. conc. ±SD	**Dissolved**	**13.5±6.8**	**2.6±1.4**	**5.7±2.7**	**16.1±19.7**	**144.2±169.9**	**6.0±1.3**
	Suspended	**3.6±3.3**	**0.5±0.7**	**0.9±2.1**	**3.4±6.3**	**40.0±89.3**	**0.7±2.5**
	Total	**17.1±8.5**	**3.1±1.6**	**6.6±4.1**	**19.5±24.8**	**184.2±224.0**	**6.7±2.6**

There are significant correlations between Cd-Cu and Zn-Ni concentrations in dissolved form in rainwater at the 95% confidence level (Figure 4.1). Statistically significant relationships between Pb-Cu, Pb-Zn, Pb-Ni, and Zn-Ni total rainwater concentrations also exist (Figure 4.2). Strong relationships between these heavy metals indicate that they undergo similar reactions in the atmosphere before they were brought down by the raindrops.

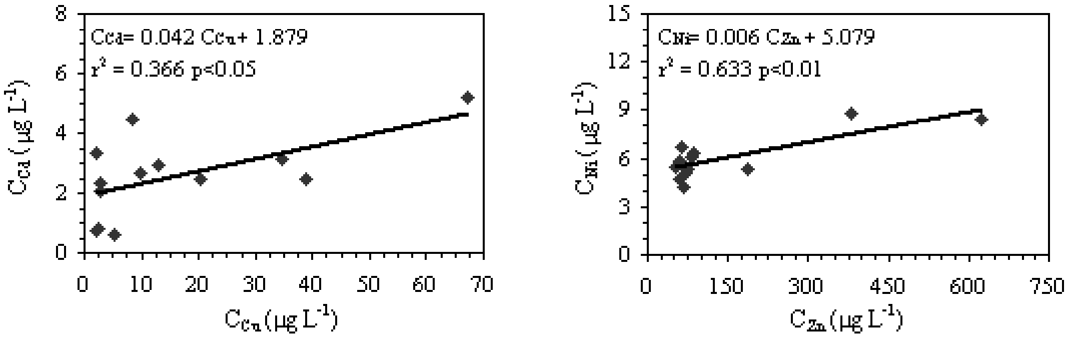


Figure 4.1 Correlations between dissolved concentrations of metals in wet deposition samples.

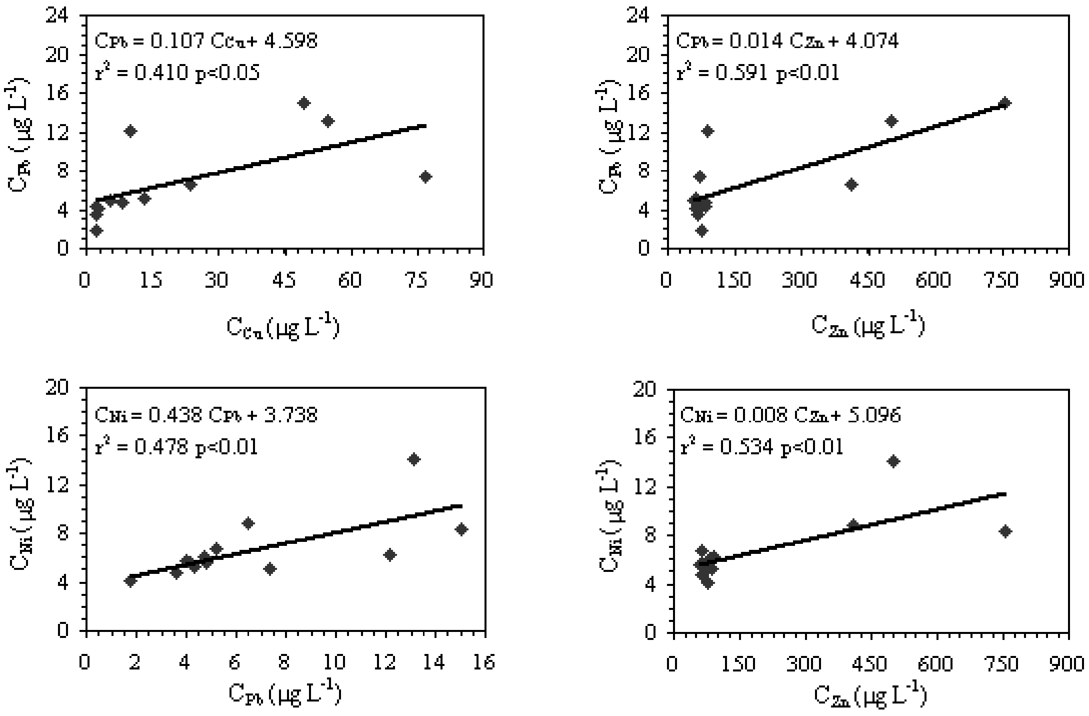


Figure 4.2 Correlations between total concentrations of metals in wet deposition samples.

Heavy metal concentrations in the rainwater samples are plotted in Figure 4.3 which shows the distribution of concentrations in dissolved, suspended and total forms for the 2003-2004 rainy periods. The ratio between the heavy metal concentrations in dissolved and suspended forms has strong fluctuations as may be noted from this figure. In this study the ratio of insoluble to soluble fractions is quite high for the first two samples for all heavy metals except for Cd. However, it must be noted that the first two samples were taken during rain incidences following elongated dry periods. This is in parallel with the results of Jaradat et al. (1999) who found that high concentrations were recorded after a dry period and when rain continued for several days concentrations were lower in rain in Amman, Jordan.

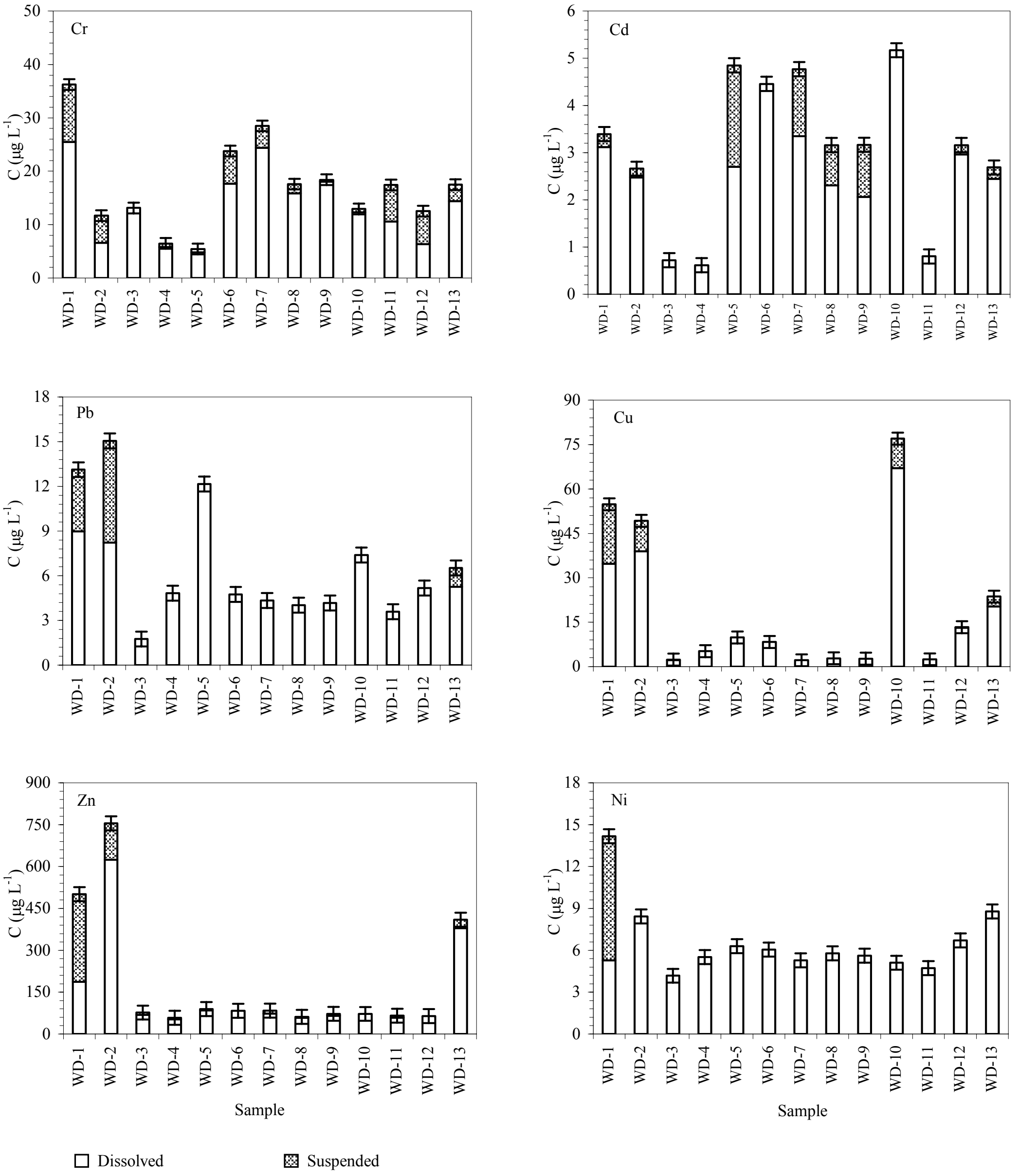


Figure 4.3 Dissolved and suspended concentrations of heavy metals in rainwater samples.

4.1.2.1 Discussion of Metal Ion Concentrations in Wet Deposition Samples

When wet deposition (rainwater) concentrations are compared with similar measurements in precipitation such as cited by Takeda, Marumoto, Minamikawa, Sakugawa, & Fujiwara (2000), Zn and Cd concentrations in the rain in Izmir are found to be higher by two orders and one order of magnitude, respectively (Table 2.2). These authors cited Zn concentration of 4.77 μg L^{-1} and Cd concentration of 0.063 μg L^{-1} on the average in the precipitation in Hiroshima, Japan. Average lead concentrations in the Izmir rainwater were 6.7 μg L^{-1} but within the range of 0.02-25.12 μg L^{-1} given by the same authors. In this comparison one must keep in mind that the average annual precipitation for the October 2003 to June 2004 rainy season in Izmir was 406.8 mm yr^{-1} which was quite below the precipitation rate of 1433 mm yr^{-1} given for Hiroshima by Takeda et al. (2000).

Izmir rainwater contained one order of magnitude more Cd, Cu, Zn and about two times more Pb than the rural station studied in western coast of France (Deboudt et al., 2004). Although the rain in Izmir Cd and Zn were higher in concentration, all other heavy metal concentrations were of the same order of magnitude as in Singapore (Hu & Balasubramanian, 2003). On the other hand, similarly high heavy metal concentrations and even higher Zn levels in the rainwater in Amman were reported by Jaradat et al. (1999). High Pb concentration of 102.0 μg L^{-1} and Ni concentration of 9.0 μg L^{-1} on the average in the precipitation in London, England were determined by Nouri, Alloway, & Peterson (2001). Cu concentrations in rainwater were reported by Kanellopoulou (2001) in Athens, Greece at the same levels as in Izmir. Trace metal concentrations in atmospheric precipitation samples collected in Hungary were in the 0.1–50 μg L^{-1} range for Cd, Co, Cu, Fe, Mn, Ni, Pb, Ti, V and Zn (Horváth, Lástity, Varga, Mészáros, & Molnár, 1994).

pH values of the rainwaters measured both at the beginning and following the wet deposition sampling were ranging between 5.1 and 7.7 with an average of 6.5. No acid rain (*i.e.* rain with $pH<5.0$) occurred during the sampling period. Yet, no significantly low pH was notable in the samples. This indicates that strongly acidic rain phenomena did not exist in Izmir during the study period. This is in contrast to the high quantity of SO_2 emissions in the area (Dincer, Muezzinoğlu, & Elbir, 2003), but in parallel with the findings of Al-Momani et al. (1995) who have concluded that the neutralizing capacity of aerosols of crustal origin in precipitation are high due to the excessive limestone coverage in the Izmir area. Al-Momani et al. (1998) have also indicated that the excessive alkaline material in crustal aerosols in Eastern Mediterranean region has a strong neutralizing impact on the rainwater. Similarly, Gülsoy, Tayanç, & Ertürk (1999) indicated the frequency of acidic pH to be about one fifth of the total rain incidences in 1996 in Istanbul, Turkey. Also, Akkoyunlu & Tayanc (2003) showed that although the SO_4^{2-} concentration in precipitation was high, so was the Ca^{2+} as a neutralizing factor of the acidity in rain. Tuncer, Bayar, Yesilyurt, & Tuncel (2001) determined that the approximately 95% of the acidity in collected precipitation samples was neutralized, particularly in summer season, and the neutralizing agents were primarily $CaCO_3$ and NH_3 in Central Anatolia.

Figure 4.4 shows the variation of average total heavy metal concentrations in wet deposition samples for the sampling period. Total concentrations of heavy metals increased according to Cd<Pb<Ni<Cr<Cu<Zn. Total concentrations of heavy metals in molar units however, indicate that Cd<Pb<Ni<Cu<Cr<Zn in quantity. Among heavy metals in rainwater samples, Zn had the highest and Cd the lowest level in this study. Relative abundance of Zn was 70%. The relative abundances of Cr and Cu were 12% and 8%, respectively. The three remaining elements (Pb, Ni, and Cd) have exhibited relative abundances below 4%.

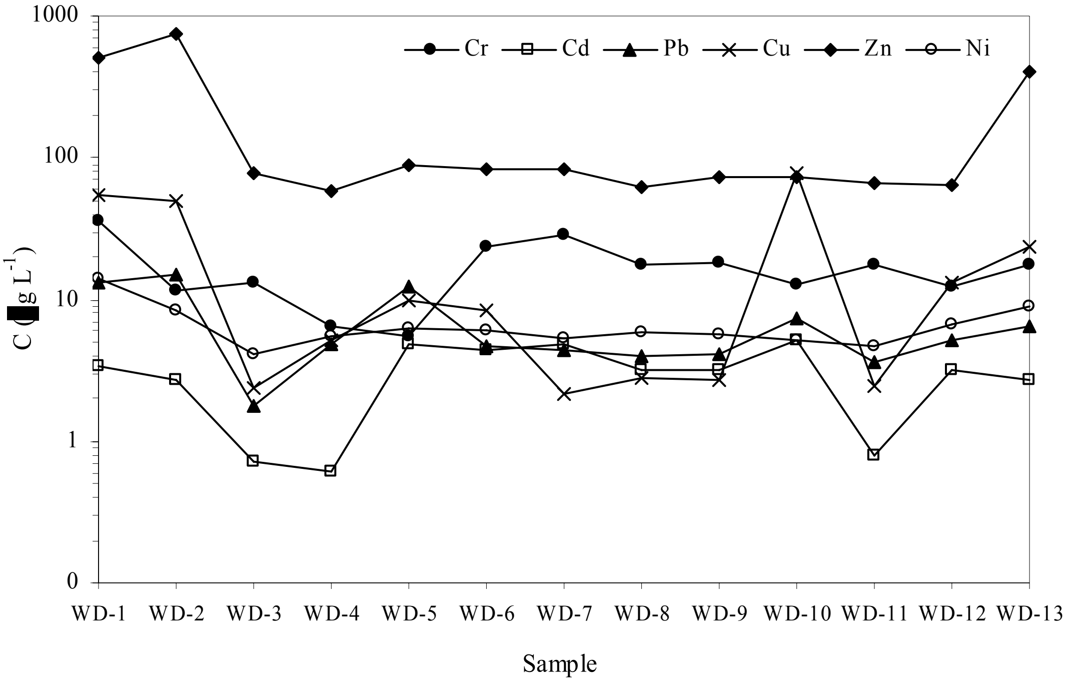


Figure 4.4 Variation of heavy metal concentrations in rainwater samples.

Statistical relationships were investigated between meteorological parameters and metal concentrations in rainwater samples. There were statistically significant relationships between the total rainwater concentrations of Pb, Zn, and Ni with air temperature at the 95% confidence level (Table 4.5).

Table 4.5 Relation between total concentrations of rainwater samples and air temperature values on sampling days (significant relationships are highlighted)

Statistical Relationship		**Heavy Metals in Wet Deposition Samples**					
		Cr	**Cd**	**Pb**	**Cu**	**Zn**	**Ni**
Total concentration-Temperature	r^2	0.181	-0.092	***0.707***	0.423	***0.863***	***0.834***
	p	>0.05	>0.05	***<0.01***	>0.05	***<0.01***	***<0.01***

Table 4.6 shows the statistically strong relationships between the total rainwater concentrations of Pb, Zn, and Ni and wind speeds at the 95% confidence level.

Table 4.6 Relationship between total concentrations of metals in rainwater samples and wind speeds during sampling

Statistical Relationship		Heavy Metals in Wet Deposition Samples					
		Cr	**Cd**	**Pb**	**Cu**	**Zn**	**Ni**
Total concentration-Wind speed	r^2	-0.034	-0.045	***0.778***	0.470	***0.811***	***0.620***
	p	>0.05	>0.05	***<0.01***	>0.05	***<0.01***	***<0.05***

Also the dissolved and total heavy metal concentrations in wet deposition samples were found uncorrelated with the rain volumes on the sampling days. This is in contrast to the results of Hou, Takamatsu, Koshikawa, & Hosomi (2005) who reported that the metal concentrations in rainwater were negatively correlated with the volume of rainwater, indicating that washout was the main mechanism that incorporates metals into rainwater. The statistically significant relationships were not found between rainy period on sampling day with dissolved and total heavy metal concentrations in rainwater samples.

4.1.3 Dissolved and Suspended Fractions of Environmental Samples

Heavy metals in atmospheric deposition may create ecotoxic effects in the receiving water and soil environments. But they are bioavailable only if the metal is in soluble form. The percentage of dissolved metals in the deposited matter depends largely on the anions with which they are incorporated. Hydrolysis rates of the heavy metal salts in the water bodies and soil waters also depend on the anion and cation balance, redox potential and pH of these waters, as well as the size distribution and chemical nature of the depositing particles (Morselli et al., 2004).

To discuss the solubility of the deposited heavy metal salts, the percentage mass ratio between the dissolved fraction and total heavy metals in dry and wet deposition were calculated. The ratios of soluble metals to the total amounts coming from atmosphere into the environmental waters may depend upon the particle size and shape, molecular form, chemical speciation and the environmental conditions. For example smaller sized particles with longer atmospheric residence times provide higher opportunities for contributions from several different emission sources and may enhance the atmospheric reactions changing the metal speciation (Voutsa & Samara, 2002). Heal, Hibbs, Agius, & Beverland (2005) have reported that a higher proportion of water-soluble metal was indicative of anthropogenic rather than crustal sources.

Water pH is another important factor determining the ratio of dissolved and suspended heavy metal fractions. Therefore, it is important to know the probability of acid rain to estimate the possibility of high hydrolysis rates of the metals. The effect of pH is the highest on moderately soluble elements most of which have anthropogenic sources. Crustal components of an element in the atmospheric particles are less soluble than the anthropogenic components. Because of these solubility differences, elements are found to be more soluble in urban aerosols than in rural aerosols which are under the influence of crustal material. Since atmospheric concentrations of anthropogenic elements such as, Cr, Pb, Cd, Zn, etc., are dominated by anthropogenic components in urban areas, the difference in the solubility of a given element in anthropogenic and crustal components does not play a significant role in the overall solubility of that element in precipitation (Kaya & Tuncel, 1997).

Values of WSS water measured both before and after daily dry deposition sampling were given in Table 3.1. The relationship between two data sets was investigated by paired t-test which shows that there is no significant difference among these data sets. In addition to that correlation coefficients were determined as $r^2=0.937$ ($p<0.01$). This indicated that the pH did not have a significant lowering during the sampling.

Neutral pH values were found in the dry deposition water samples (5.6-7.8 with an average of 6.6) before and after the daily sampling and also in the rainwater samples (5.1-7.7 with an average of 6.5). A significance test was applied to discuss the impact of pH on the solubility of the heavy metals. It may be followed that no pH lowering during sampling was notable in any sample to create changes in solubilities.

In fact no pH lowering was notable in any sample which would create changes in solubility of the metals.

Dissolved and suspended fractions of dry and wet deposition samples are shown in Figure 4.5 and Figure 4.6. Although the average figures in this study are in parallel with the results in literature, the ratio of dry and wet deposition heavy metal concentrations in dissolved and suspended depositions showed stronger fluctuations. The ratio of insoluble to soluble fractions is quite high for the first two rain samples for all heavy metals except for Cd. It must be noted that the first two samples are taken during rain incidences following elongated dry periods.

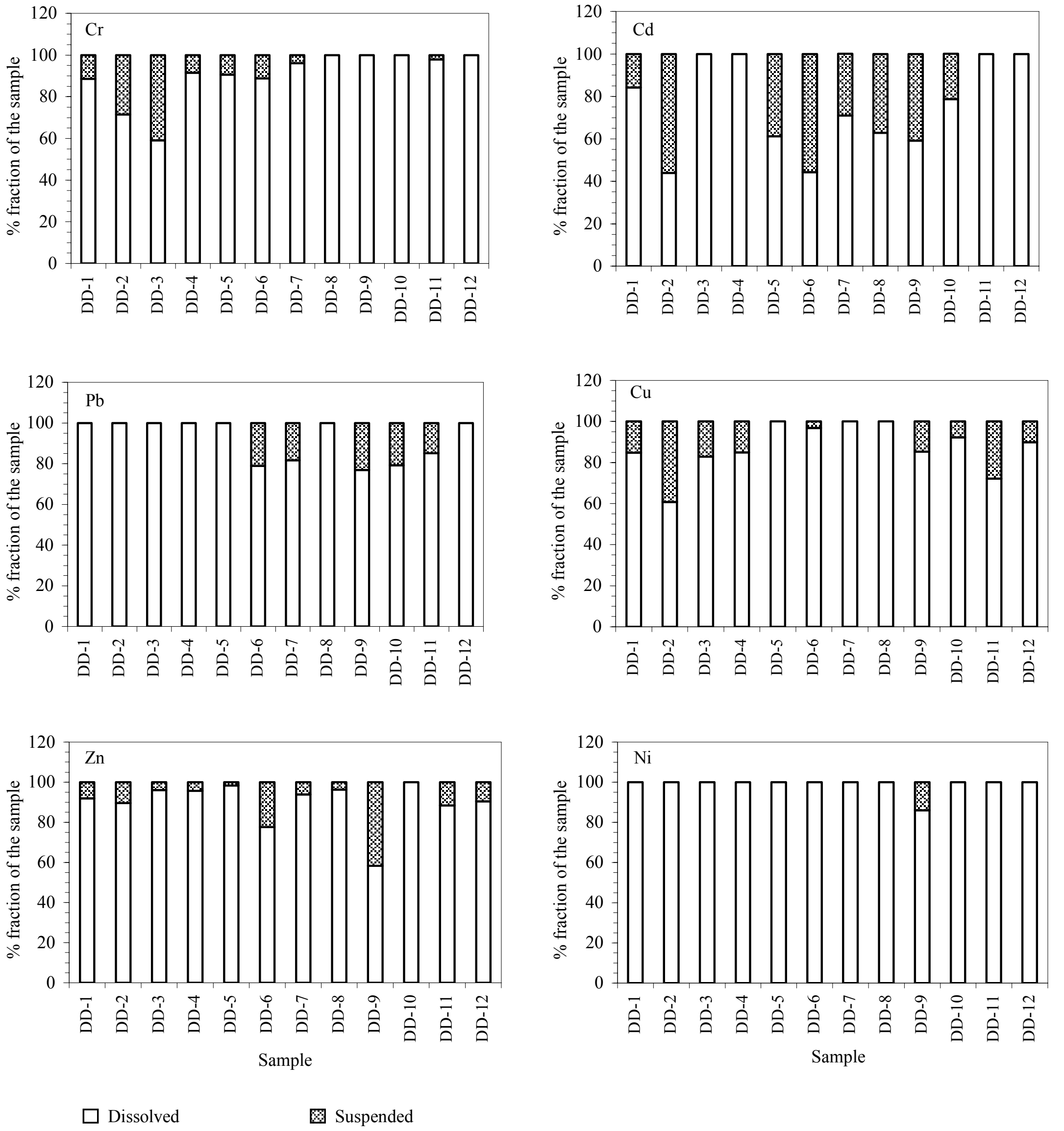


Figure 4.5 Dissolved and suspended fractions for heavy metals of dry deposition samples.

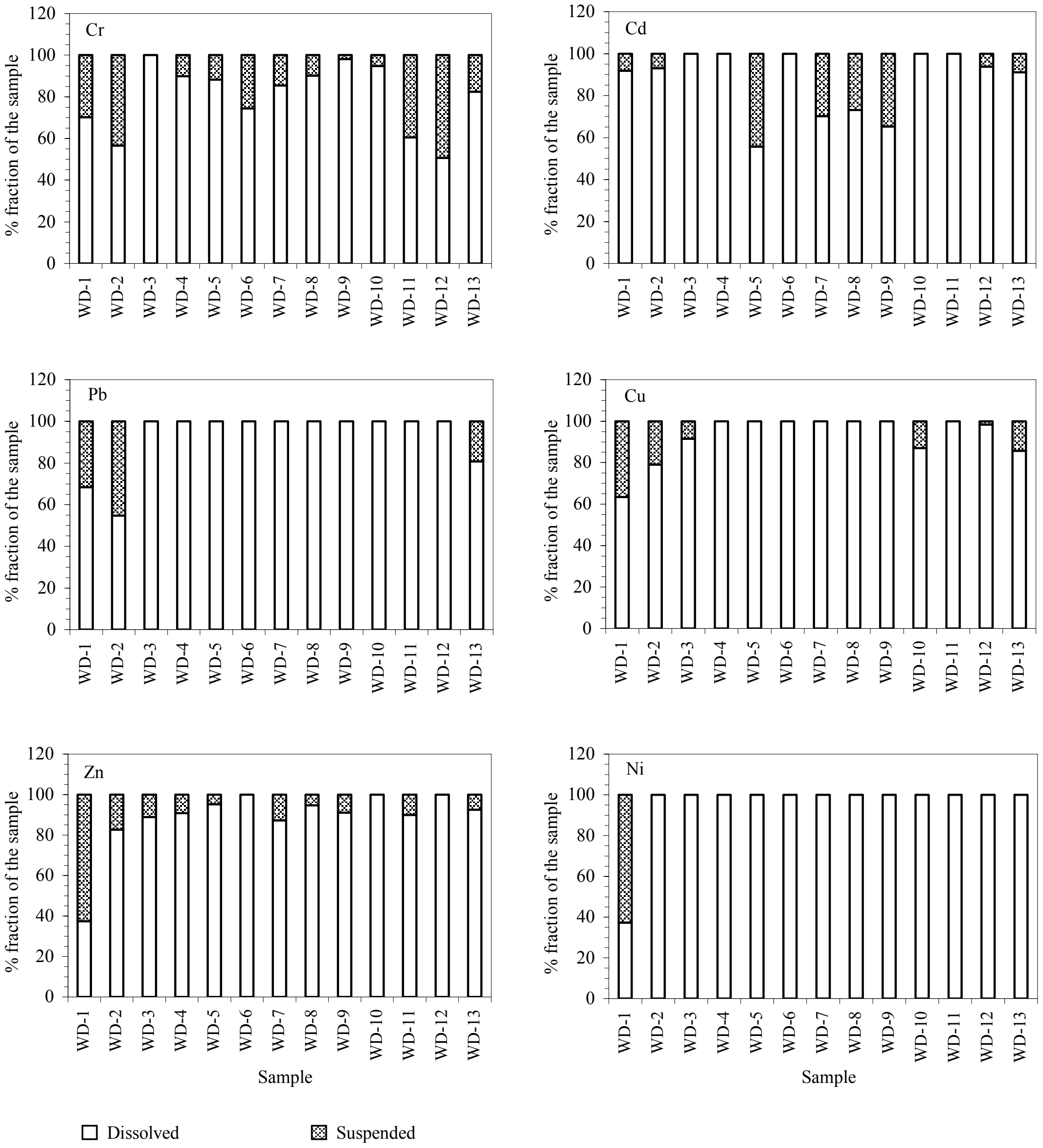


Figure 4.6 Dissolved and suspended fractions for heavy metals of rainwater samples.

The soluble average percentages of the Cr, Cd, Pb, Cu, Zn and Ni in dry deposition samples are given in Table 4.7. The ratios of soluble fractions of heavy metals in the dry deposition are within 44-100 percent range of the total metals in deposition except for one or two samples having somewhat less soluble Cd and Cr forms in deposition. This result is in contradiction with the results of Morselli, Olivieri, Brusori, & Passarini (2003) who reported lower soluble fractions in dry deposition than in wet deposition in Bologna, Italy. In contrast to this, Voutsa & Samara (2002) have reported higher soluble fractions of the Cd, Pb, Cu, Zn and Ni in the Greater Thessaloniki area of Greece. Significantly higher labile fractions of these metals were found suggesting the dominance of easily available metal forms in atmospheric particles.

Table 4.7 Soluble fractions of the dry deposited heavy metals

Reference	Place	Soluble Fraction in Dry Deposition, %					
		Cr	Cd	Pb	Cu	Zn	Ni
Heal et.al., 2005	Edinburgh, UK (urban)	-	50.0	35.0-45.0	35.0-45.0	50.0	≤10
Morselli et al., 2003	Bologna, Italy (urban)	-	33.0	-	-	25.0	-
Voutsa & Samara, 2002	G.Thessaloniki, Greece (urban/industrial)	-	80.0	66.0	90.0	93.0	82.0
This study	Izmir, Turkey (suburban/traffic)	90.4	75.4	91.8	87.5	89.7	98.8

Table 4.8 shows the soluble average percentages of the heavy metals in rainwater samples. The ratios of soluble fractions of heavy metals in the rainwater are within 37-100 percent range of the total metals in deposition except for one or two samples having somewhat less soluble Cd and Cr forms in deposition. Another exception to that is Ni which has a larger range of soluble fractions in wet deposition than in dry deposition. Kaya & Tuncel (1997) have reported lower solubilities for heavy metals in Ankara rainwater than our results except for Cd. However, the results reported by Morselli et al. (2003) were similar to our solubility values in wet deposition samples. The solubility of Cu, in rainwater samples that sampled in Büyükçekmece Lake, Istanbul, was found as 8.4% by Başak & Alagha (2004).

Several studies have reported solubilities of trace elements in rainwater by analyzing the filtrate (soluble fraction) and filter (insoluble fraction), respectively (Kaya & Tuncel, 1997; Golomb et al., 1997). In these reports, solubilities of Al and Fe were low, 4-25%, whilst those of Cd, Cu, Mn, Pb, V, and Zn were higher than 40%. In our study, however, rain samples were filtered immediately after sampling and right before acidifying the samples, and then the soluble fraction of trace elements in precipitation were determined. In most of previous data, however, samples were acidified before filtration, and insoluble and soluble fractions did not correspond to their precise definitions. Therefore, in comparing the average concentrations in this study with those reported previously, this difference in sample pretreatment must be taken into consideration.

Table 4.8 Soluble fractions of the wet deposited heavy metals

Reference	Place	Soluble Fraction in Wet Deposition, %					
		Cr	**Cd**	**Pb**	**Cu**	**Zn**	**Ni**
Özsoy & Örnektekin, 2005	Iskenderun, Turkey (urban/industrial)	20.1	52.3	46.1	43.4	77.7	43.9
Morselli et al., 2003	Bologna, Italy (urban)	88.0	68.0	61.0	-	74.0	-
Kaya & Tuncel, 1997	Ankara, Turkey (urban)	35.0	88.0	40.0	49.0	43.0	72.0
This study	Izmir, Turkey (suburban/traffic)	80.1	87.2	92.6	92.7	88.5	95.2

This discussion in relation to the soluble fraction ratios in deposition in Izmir indicated that the studied metals are ready to impose ecotoxic impacts in the water-soil environments and on biota. This impact is enhanced by highly soluble fractions in the dry deposition, too. Sudden increases in heavy metal deposition in dissolved form can be an important input into the biochemical cycles and may create more significant impacts.

The results of this study underline the importance of metal pollution in the rainwater especially in regard to the ecotoxic properties of heavy metals rather than the dry deposition which occurs on the long run. It also showed that the pH is a relatively unimportant property in creating ecotoxic effects due to enhanced solubility.

4.2 Dry and Wet Deposition Fluxes of Environmental Samples

Dry and wet deposition fluxes of dry deposition and rainwater samples were calculated and the results of heavy metal dry and wet deposition measurements are presented in this section.

4.2.1 Dry Deposition Fluxes

Average daily dry deposition heavy metal fluxes for the dissolved, suspended and total fractions are shown in Table 4.9. The measured and calculated average total dry deposition fluxes for Cr, Cd, Pb, Cu, Zn, and Ni were 96.4±69.2, 41.3±20.0, 90.3±40.8, 81.3±48.6, 2127.2±651.4, and 139.6±62.0 µg m^{-2} day^{-1}, respectively.

The average dry deposition of Zn was the highest and the average Cd flux was the lowest of the metal fluxes. This is in good agreement with the results reported by previous studies (Herut, Nimmo, Medway, Chester, & Krom, 2001; Migon et al., 1997; Pirrone et al., 1995; Sakata & Marumoto, 2004).

Table 2.4 indicates that the dry deposition fluxes were appreciably higher in Izmir compared to elsewhere. For example, dry depositions found in this study were 10-50 times higher for Cd; 4-20 times higher for Pb and 5-30 times higher for Zn than the results of a study based on WSS in Tokyo, Japan (Sakata & Marumoto, 2004). The average measured Pb, Cu, and Zn fluxes were found as 0.07, 0.06, and 0.20 mg m^{-2} day^{-1} in Chicago; 0.003, 0.01 and 0.01 mg m^{-2} day^{-1} over Lake Michigan; and 0.004, 0.007 and 0.004 mg m^{-2} day^{-1} in South Haven, respectively (Sofuoglu & Holsen, 1997).

Table 4.9 Dry deposition fluxes of WSS water samples

Sample		**Dry Deposition Fluxes (μg m^{-2} day^{-1})**					
		Cr	**Cd**	**Pb**	**Cu**	**Zn**	**Ni**
DD-1	Dissolved	50.9	49.2	112.5	43.3	2527.2	184.8
	Suspended	6.6	9.3	nd	7.8	224.3	nd
	Total	57.5	58.5	112.5	51.1	2751.5	184.8
DD-2	Dissolved	46.1	14.7	33.7	23.8	2190.1	58.2
	Suspended	18.5	18.8	nd	15.3	254.6	nd
	Total	64.6	33.5	33.7	39.1	2444.7	58.2
DD-3	Dissolved	142.4	18.8	67.9	47.5	1566.0	190.8
	Suspended	98.4	nd	nd	6.0	64.3	nd
	Total	240.8	18.8	67.9	53.5	1630.3	190.8
DD-4	Dissolved	99.1	17.7	68.4	43.1	2622.9	167.4
	Suspended	9.2	nd	nd	7.5	119.3	nd
	Total	108.3	17.7	68.4	50.6	2742.2	167.4
DD-5	Dissolved	51.5	33.4	69.8	201.7	2914.1	90.1
	Suspended	5.4	21.2	nd	nd	46.6	nd
	Total	56.9	54.6	69.8	201.7	2960.7	90.1
DD-6	Dissolved	163.5	30.2	137.6	101.4	1050.8	238.3
	Suspended	20.6	38.0	36.9	3.4	301.8	nd
	Total	184.1	68.2	174.5	104.8	1352.6	238.3
DD-7	Dissolved	109.4	31.9	52.5	53.5	1949.8	78.4
	Suspended	2.7	13.1	11.8	nd	127.4	nd
	Total	112.1	45.0	64.3	53.5	2077.2	78.4
DD-8	Dissolved	38.5	36.1	72.6	60.3	2358.7	93.7
	Suspended	nd	21.4	nd	nd	63.6	nd
	Total	38.5	57.5	72.6	60.3	2422.3	93.7
DD-9	Dissolved	43.8	12.5	58.6	68.4	435.3	83.6
	Suspended	nd	8.7	17.6	12.0	311.8	13.9
	Total	43.8	21.2	76.2	80.4	747.1	97.5
DD-10	Dissolved	39.3	52.8	89.9	71.2	2190.1	224.8
	Suspended	nd	14.3	23.7	6.0	nd	nd
	Total	39.3	67.1	113.6	77.2	2190.1	224.8
DD-11	Dissolved	172.7	14.2	131.8	39.1	2178.7	162.6
	Suspended	3.7	nd	23.1	15.8	284.3	nd
	Total	176.4	14.2	154.9	54.9	2463.0	162.6
DD-12	Dissolved	34.5	38.7	75.4	134.2	1578.5	88.3
	Suspended	nd	nd	nd	14.7	166.6	nd
	Total	34.5	38.7	75.4	148.9	1745.1	88.3
Average flux ±SD	**Dissolved**	**82.6± 52.4**	**29.2± 13.7**	**80.9± 31.6**	**74.0± 50.2**	**1963.5± 703.0**	**138.4± 63.0**
	Suspended	**13.8± 27.6**	**12.1± 11.7**	**9.4± 12.9**	**7.4± 6.0**	**163.7± 109.2**	**1.2± 4.0**
	Total	**96.4± 69.2**	**41.3± 20.0**	**90.3± 40.8**	**81.3± 48.6**	**2127.2± 651.4**	**139.6± 62.0**

Dry deposition fluxes are summarized in the form of dissolved and suspended fractions of heavy metals in Figure 4.7. The standard deviations around the average total metal fluxes are indicated as error bars. The dry deposition heavy metal fluxes were rather high with high fluctuations throughout the study period.

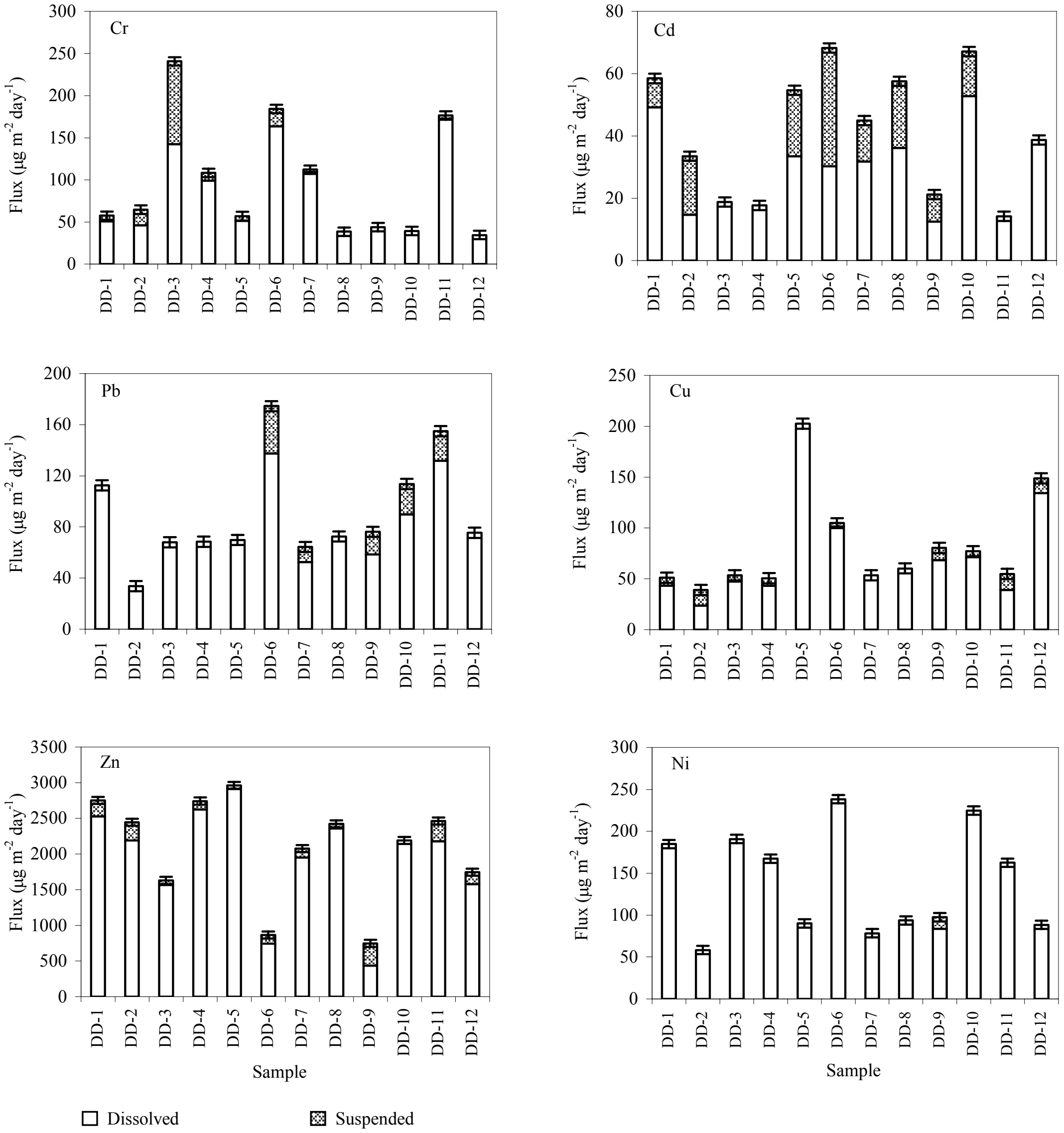


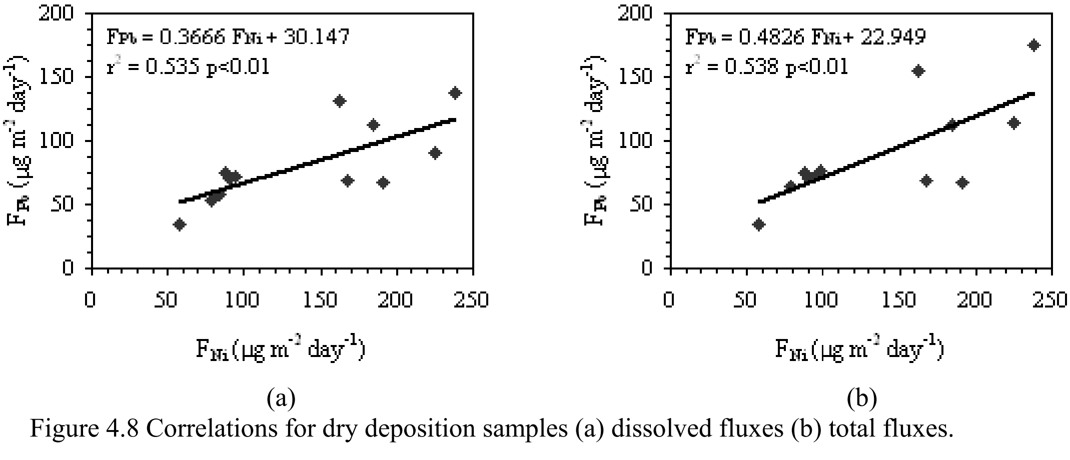
Figure 4.7 Dry deposition fluxes of heavy metals in dry deposition samples.

Statistical evaluations shows that the sticky deposition plate and water surface sampler methods give the same dry deposition results, although the sampling methods are different (Odabasi et al., 1999; Sakata & Marumoto, 2004). Therefore, the dry deposition data generated in this study may be compared with the older data at the same study site in Izmir (Odabasi et al., 2002). Within the two years time in between the studies, the total Cr, Cd have increased; Cu, Zn and Ni did not show a significant change whilst there has been a decrease in the average value of Pb fluxes in the dry deposition at the study site. Also the range of the lead fluxes became narrower thus the maximum value approached the average flux data obtained two years ago. At the beginning of 2004 leaded gasoline has been largely withdrawn from the market and this might perhaps be a first indication of the positive impact on the environmental quality of minimized use of the unleaded gasoline in the area. The higher dry deposition fluxes of the studied metals presumably come from the soil components as the sampler was only 3 meters to the ground. Odabasi et al. (2002) showed older pollution in the soil composition which may cause re-entrainment of metals in the air and contribute to the deposition in the study site.

When we relate the dry deposition heavy metal fluxes with corresponding wind directions, it is notable that higher depositions occurred when it blew from the north. On the contrary lower fluxes occurred with southerly winds.

There was no statistically significant relationship between dissolved and total fluxes of heavy metals in dry deposition samples and meteorological parameters (wind speed, air temperature, and relative humidity) at the 95% confidence level.

Strong relationships were not found between heavy metal fluxes in dry deposition samples when total and soluble fractions were separately tested; highest correlations were found between the Pb and Ni soluble and total flux fractions, respectively (Figure 4.8). This indicates that these metals have a common source, possibly of traffic origin at the study site.



(a) (b)

Figure 4.8 Correlations for dry deposition samples (a) dissolved fluxes (b) total fluxes.

Figure 4.9 shows the dry deposition fluxes of the anthropogenic trace metals measured over the sampling period in Izmir. As shown in Figure 4.9 the dry deposition fluxes for trace metals increased according to Cd<Cu<Pb<Cr<Ni<Zn for almost all sampling periods. This range is in good agreement with the results reported by Tasić et al. (2001) and Yun et al. (2002).

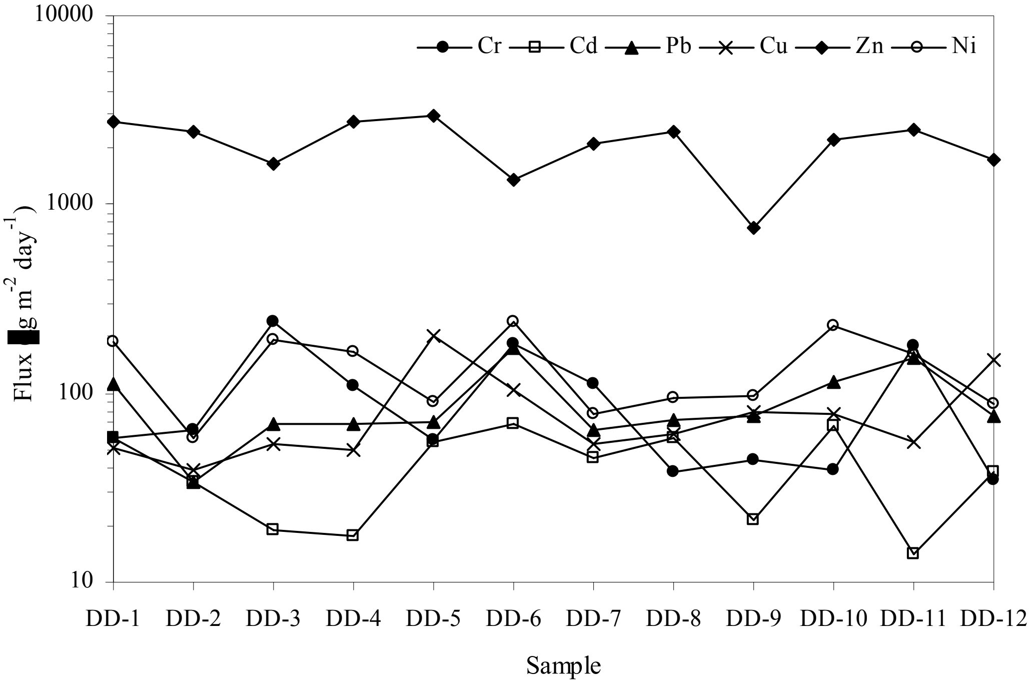


Figure 4.9 Changes in total dry deposition fluxes for heavy metals (October 2003 to June 2004).

There was no significant difference between the deposition fluxes of metal between seasons in this study. Likewise no seasonal trends in total particle mass heavy metals were reported by Sakata & Marumoto (2004) and Sweet et al. (1998) at their respective study sites.

Azimi et al. (2005) reported that these elements were mostly (>95%) of non-crustal origin. Bozlaker (2002) gives the composition of Cd, Zn, Pb, and Ni in dry deposition indicating their dominant anthropogenic origin exhibited by their high EF_{crust} values (>10) at the same site as this study. Particles originating from resuspension of dust, which is an important source of many trace metals (Sweet et al., 1998), have an important influence on atmospheric deposition (Azimi et al., 2005).

4.2.2 Wet Deposition Fluxes of Rainwater Samples

Average daily wet deposition heavy metal fluxes for the dissolved, suspended and total fractions of samples are shown in Table 4.10. The measured average total wet deposition fluxes for Cr, Cd, Pb, Cu, Zn, and Ni were 271.2±148.1, 54.2±46.1, 111.5±77.9, 362.6±670.5, 2387.7±2807.2, and 107.8±49.7 µg m^{-2} day^{-1}, respectively. Zn has highest daily wet deposition fluxes; Cd has lowest daily wet deposition fluxes in this study. This is an agreement with the results from reported by the previous studies (Tasić et al., 2001; Migon et al., 1997).

Table 4.11 summarizes the wet deposition fluxes with other places in the world. From this table it may be noted that the wet deposition fluxes were appreciably high in Izmir. This difference may originate from the regional climate characteristics, types proximity of pollution sources, differences of sampling methods, precipitation amount, and period of rainy season. Also the definition of flux per day is obscure in the reference research mentioned in the table. From the Table 4.12 it may be noted that the wet deposition fluxes which calculated according to this formula were also appreciably high in Izmir.

Table 4.10 Wet deposition fluxes of rainwater samples

Sample		**Wet Deposition Fluxes (μg m^{-2} day^{-1})**					
		Cr	**Cd**	**Pb**	**Cu**	**Zn**	**Ni**
WD-1	Dissolved	211.0	25.8	74.4	287.9	1551.0	43.8
	Suspended	89.1	2.3	34.3	166.3	2596.6	73.6
	Total	300.1	28.1	108.7	454.1	4147.6	117.4
WD-2	Dissolved	98.6	36.9	122.7	581.3	9313.1	125.7
	Suspended	75.6	2.8	101.7	153.1	1948.5	nd
	Total	174.2	39.7	224.4	734.4	11261.6	125.7
WD-3	Dissolved	217.4	12.0	29.1	35.8	1138.1	69.2
	Suspended	nd	nd	nd	3.3	142.0	nd
	Total	217.4	12.0	29.1	39.1	1280.1	69.2
WD-4	Dissolved	103.0	10.9	85.5	92.6	937.2	97.7
	Suspended	11.6	nd	nd	nd	94.2	nd
	Total	114.6	10.9	85.5	92.6	1031.4	97.7
WD-5	Dissolved	92.7	52.1	234.8	190.7	1640.6	121.9
	Suspended	12.3	41.6	nd	nd	82.5	nd
	Total	105.0	93.7	234.8	190.7	1723.1	121.9
WD-6	Dissolved	183.3	46.2	49.2	86.4	865.7	62.6
	Suspended	63.0	nd	nd	nd	nd	nd
	Total	246.3	46.2	49.2	86.4	865.7	62.6
WD-7	Dissolved	269.1	37.0	47.9	23.8	812.1	58.3
	Suspended	45.5	15.7	nd	nd	118.9	nd
	Total	314.6	52.7	47.9	23.8	931.0	58.3
WD-8	Dissolved	511.1	74.4	129.7	90.6	1887.9	186.4
	Suspended	55.7	27.5	nd	nd	106.2	nd
	Total	566.8	101.9	129.7	90.6	1994.1	186.4
WD-9	Dissolved	232.5	26.6	53.8	34.7	847.2	72.5
	Suspended	4.6	14.2	nd	nd	83.0	nd
	Total	237.1	40.8	53.8	34.7	930.2	72.5
WD-10	Dissolved	395.7	166.5	238.0	2160.0	2321.6	164.6
	Suspended	21.5	nd	nd	321.7	nd	nd
	Total	417.2	166.5	238.0	2481.7	2321.6	164.6
WD-11	Dissolved	272.1	20.7	92.5	63.8	1525.4	121.8
	Suspended	177.2	nd	nd	nd	170.7	nd
	Total	449.3	20.7	92.5	63.8	1696.1	121.8
WD-12	Dissolved	169.5	79.1	138.2	350.2	1726.1	179.0
	Suspended	164.9	5.3	nd	5.6	nd	nd
	Total	334.4	84.4	138.2	355.8	1726.1	179.0
WD-13	Dissolved	39.8	6.8	14.5	56.1	1048.2	24.3
	Suspended	8.4	0.7	3.5	9.3	83.6	nd
	Total	48.2	7.5	18.0	65.4	1131.8	24.3
Average flux ±SD	**Dissolved**	**215.1± 129.6**	**45.8± 42.8**	**100.8± 71.2**	**311.8± 578.5**	**1970.3± 2255.0**	**102.1± 52.6**
	Suspended	**56.1± 58.7**	**8.5± 13.0**	**10.7± 28.9**	**50.7± 100.5**	**417.4± 835.6**	**5.7± 20.4**
	Total	**271.2± 148.1**	**54.3± 46.1**	**111.5± 77.9**	**362.5± 670.5**	**2387.7± 2807.2**	**107.8± 49.7**

Table 4.11 Wet deposition fluxes of heavy metals around the world (µg m^{-2} day^{-1})

Flux						**Method**	**Location**	**Period**	**Reference**
Cr	**Cd**	**Pb**	**Cu**	**Zn**	**Ni**				
-	2.3	130.8	89.4	221.2	-	Wet/dry collector	Belgrade, Yugoslavia	April and May 1999	Tasić et al., 2001
-	0.09	1.6	1.6	106.0	1.9	Total deposition sampler	Ligurian Sea, France	October 1992 to September 1993	Migon et al., 1997

Daily wet deposition fluxes of different heavy metals in the area are summarized in Figure 4.10 indicating the range of wet deposited heavy metal fluxes found at the site for the study period. Fluctuations between individual sampling days can be noted from this figure with the dissolved and suspended heavy metal fractions in wet deposition separately shown. The standard deviations around the average total metal fluxes are indicated as error bars.

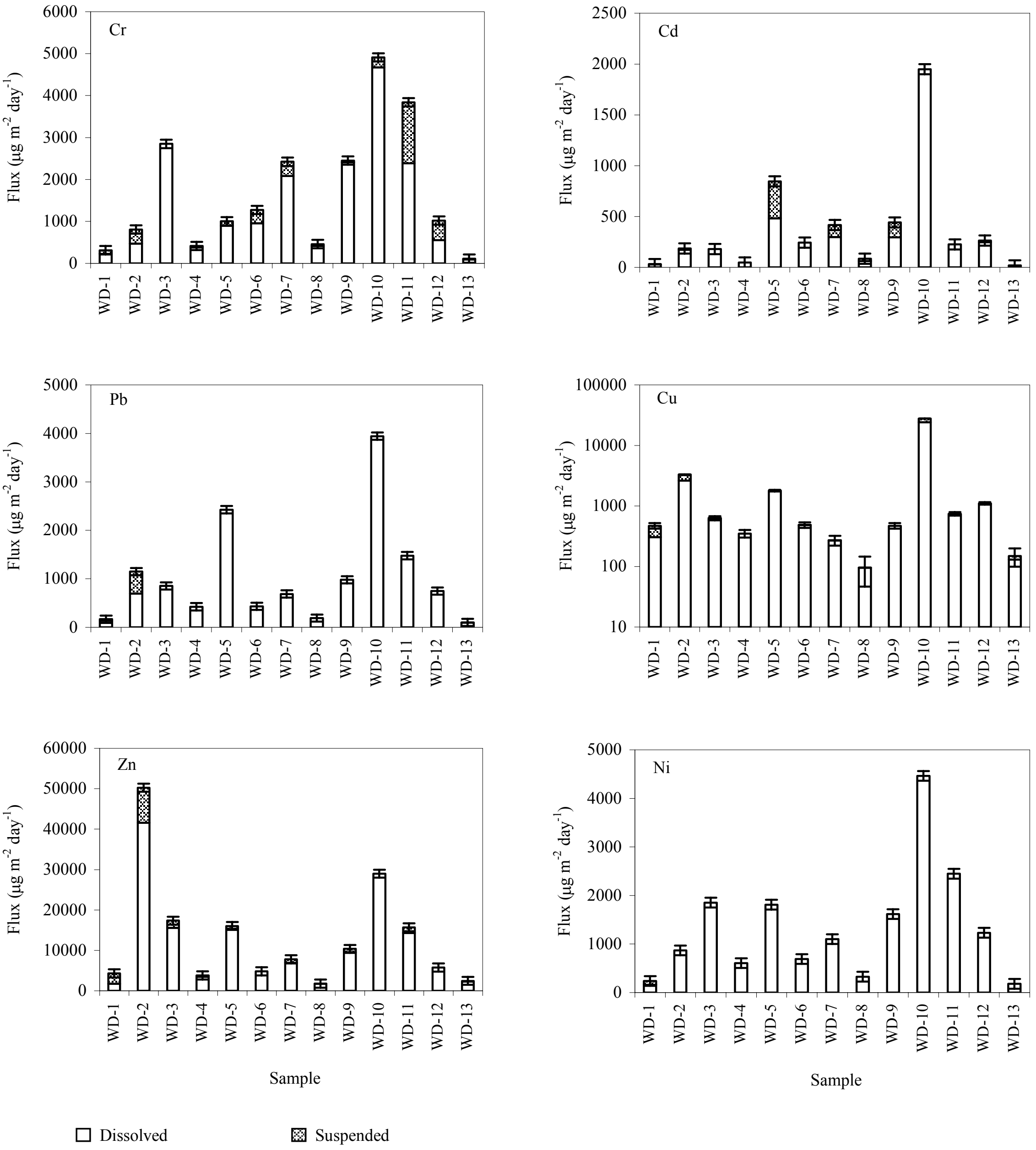


Figure 4.10 Wet deposition fluxes of heavy metals in rainwater samples.

When all of the wet deposition fluxes for heavy metals are drawn together throughout the study period Figure 4.11 is obtained. High fluctuations in wet deposition fluxes among samples are much more pronounced in comparison to the dry deposition fluxes (Figure 4.9). For wet deposition fluxes of the metals except for Cu and Zn, the lowest values were observed in June 2004. This is due to the very small amount (2.8 mm day^{-1}) of precipitation in this month. On the other hand, the highest values were observed in 22 January 2004 except for Cr and Zn. This is due to the highest precipitation (32.2 mm day^{-1}) in this month. The order of daily wet deposition flux values for the six heavy metals was Cd<Ni<Pb<Cr<Cu<Zn, which was different from the series in dry deposition.

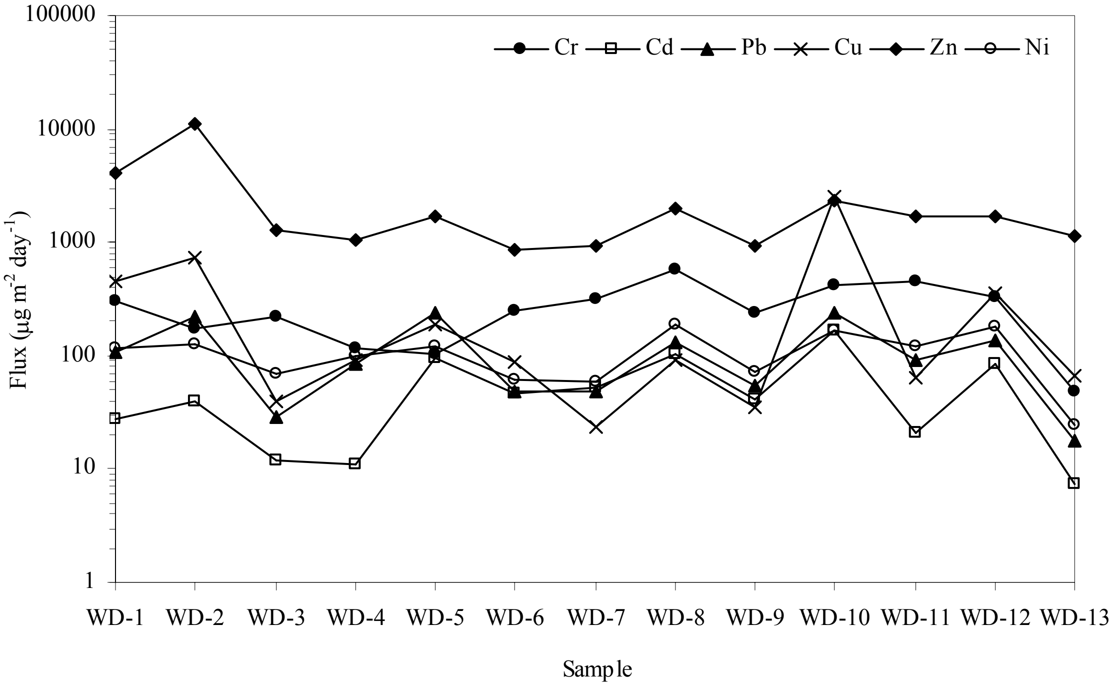


Figure 4.11 Variations in total wet deposition flux values for heavy metals (October 2003 to June 2004).

Values of r^2 and level of significance are summarized in Tables 4.12 to 4.13 in multiple correlation tests among the soluble and total metals in wet deposition fluxes. From these two tables it may be seen that the wet deposition fluxes of selected metals are interrelated except with Zn. In soluble form, most important relationship was found between Cd and Cu ($r^2=0.745$; $p<0.01$). In total form, a significant relation was found between Cd and Cu ($r^2=0.518$; $p<0.01$). Strong relationships are thought to be due to probable common sources and/or similar atmospheric reactions before they settled down by the raindrops. The correlation coefficients among the fluxes of metals are much better for wet deposition fluxes than the corresponding dry deposition fluxes.

Table 4.12 Matrix of correlation coefficients of soluble heavy metal fluxes in wet deposition samples

		Cr	Cd	Pb	Cu	Zn	Ni
Cr	r^2	1.00					
	p	0.00					
Cd	r^2	***0.318***	1.00				
	p	***<0.05***	0.00				
Pb	r^2	0.060	***0.575***	1.00			
	p	>0.05	***<0.01***	0.00			
Cu	r^2	0.105	***0.745***	***0.436***	1.00		
	p	>0.05	***<0.01***	***<0.05***	0.00		
Zn	r^2	0.026	0.010	0.066	0.078	1.00	
	p	>0.05	>0.05	>0.05	>0.05	0.00	
Ni	r^2	0.256	***0.461***	***0.551***	0.192	0.083	1.00
	p	>0.05	***<0.05***	***<0.01***	>0.05	>0.05	0.00

Table 4.13 Matrix of correlation coefficients of total heavy metal fluxes in wet deposition samples

		Cr	Cd	Pb	Cu	Zn	Ni
Cr	r^2	1.00					
	p	0.00					
Cd	r^2	0.246	1.00				
	p	>0.05	0.00				
Pb	r^2	0.023	***0.491***	1.00			
	p	>0.05	***<0.01***	0.00			
Cu	r^2	0.064	***0.518***	***0.424***	1.00		
	p	>0.05	***<0.01***	***<0.05***	0.00		
Zn	r^2	0.007	0.000	0.288	0.076	1.00	
	p	>0.05	>0.05	>0.05	>0.05	0.00	
Ni	r^2	***0.420***	***0.466***	***0.513***	0.203	0.066	1.00
	p	***<0.05***	***<0.01***	***<0.01***	>0.05	>0.05	0.00

When we compare wet deposition heavy metal fluxes with corresponding wind directions, higher values have occurred when it blew from the north mostly. On the contrary lower fluxes were found during southerly winds.

There were statistically significant relationships between wind speed values and dissolved and total fluxes of Zn ($r^2=0.779$; $p<0.01$ and $r^2=0.876$; $p<0.01$, respectively). The statistical relationships between air temperatures on sampling days and dissolved and total fluxes of Cr have been found to be $r^2=0.379$ ($p<0.05$) and $r^2=0.358$ ($p<0.05$), respectively. There was no statistically significant relationship between dissolved and total fluxes of heavy metals in wet deposition samples and relative humidity values at the 95% confidence level.

In this study, statistical relationships between daily rain volumes and daily soluble metal fluxes have been found to be $r^2=0.446$ ($p<0.05$) for Cr; $r^2=0.477$ ($p<0.01$) for Cd; $r^2=0.476$ ($p<0.01$) for Pb, and $r^2=0.875$ ($p<0.01$) for Ni. Daily rain volumes and daily total fluxes of Cr ($r^2=0.504$; $p<0.01$), Cd ($r^2=0.490$; $p<0.01$), Pb ($r^2=0.306$; $p<0.05$), and Ni ($r^2=0.745$; $p<0.01$) were correlated, too. These are in contradiction with Takeda et al. (2000) who reported that Cr, Zn, Cu, and Ni fluxes do not correlate with the amount of precipitation except for Cu and Zn but in parallel with the Pb results by Kim (1998). Sakata et al. (2006) noted that the annual wet deposition fluxes of As, Hg, Cr, Cd, Pb, Mn, V, Sb, and Se were correlated with the annual precipitation amount.

Sweet et al. (1998) stated that wet deposition of metals was more closely related to amount of precipitation than the dependence of the concentration of metals in precipitation to rain volume. The results of Luo (2001) showed that all the wet deposition fluxes of soluble chemical species and the elements in insoluble materials have strong correlations with rain intensity. Kaya & Tuncel (1997) have reported that the low wet deposition fluxes of measured parameters found in Ankara, Turkey was due to low precipitation amount in the sampling period. Tanner & Wong (2000) indicated several occasions of low rainfall amount, small rainfall intensity, and low wind speed, producing high concentrations of trace metals in bulk deposition in Hong Kong, with pH values down to 3.6.

In Izmir, the relationships between daily rain period and daily dissolved and total fluxes of heavy metals have not been found. Maneux et al. (1999) reported that although elements display generally parallel variations with time, they sometimes follow independent behaviors (e.g. Pb and Cd), suggesting that they may derive from different geographical and/or pollution sources.

4.2.3 Annual Heavy Metal Deposition Fluxes in Dry and Wet Forms

The annual dry and wet deposition fluxes of heavy metals are given in Table 4.14. The estimated quantity of atmospheric cleansing with dry deposition was more important than that of wet deposition throughout the study year of 1 October 2003-1 November 2004 in Izmir. The ratios of dry versus wet deposition per year were 4.7 times for Cr, 11.5 times for Cd, 11.1 times for Pb, 3.4 times for Cu, 9.5 times for Zn and 17.3 times for Ni. This comparison shows the relative significance of dry deposition in this Mediterranean climate area due to the low intensity and frequency of rain incidences and rather low humidity in the air.

Table 4.14 Comparison of annual heavy metal fluxes of dry and wet deposition samples (kg km^{-2} yr^{-1})

Sample Fraction		**Metal**					
		Cr	**Cd**	**Pb**	**Cu**	**Zn**	**Ni**
Dissolved flux	DD	27.6±17.5	9.8±4.6	27.0±10.5	24.7±16.7	655.8±234.8	46.2±21.0
	WD	5.5±2.7	1.0±0.5	2.3±1.1	6.6±8.0	58.7±69.1	2.4±0.5
Suspended flux	DD	4.6±9.2	4.0±3.9	3.1±4.3	2.5±2.0	54.7±36.5	0.4±1.3
	WD	1.4±1.3	0.2±0.3	0.4±0.9	1.4±2.5	16.2±36.3	0.3±1.0
Total flux	DD	32.2±23.1	13.8±6.7	30.1±13.6	27.2±16.2	710.5±217.6	46.6±21.3
	WD	6.9±3.5	1.2±0.6	2.7±1.7	8.0±10.1	74.9±91.1	2.7±1.1

The dry and wet total deposition fluxes on annual base are shown in Figure 4.12. The annual dry deposition fluxes were found larger than the annual wet deposition fluxes. Sakata & Marumoto (2004), Azimi et al. (2003), and Migon et al. (1997) stated that dry deposition is the dominant mechanism of the heavy metals deposition. Figure 4.13 shows that the daily wet deposition fluxes were larger than the dry deposition fluxes by 2.8 times for Cr, 1.3 times for Cd, 1.2 times for Pb, 4.5 times for Cu, 1.1 times for Zn and 0.8 times for Ni. Wet deposition rates except Ni are more significant than the dry deposition rates which may on a daily basis.

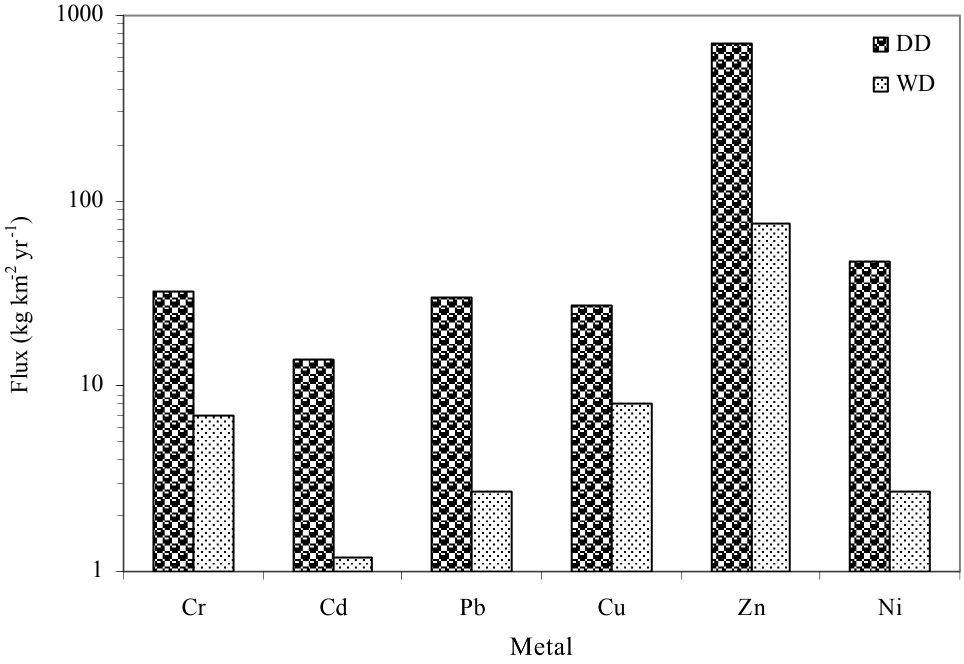


Figure 4.12 Comparison between annual dry and wet deposition fluxes for heavy metals.

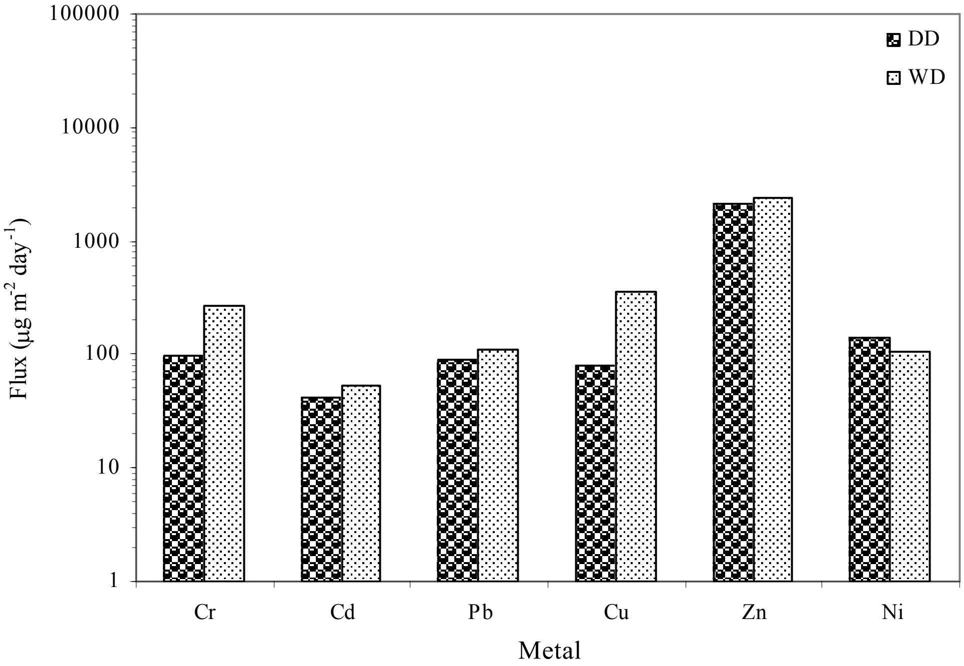


Figure 4.13 Comparison between daily dry and wet deposition fluxes for heavy metals.

Annual total (wet+dry) fluxes of Cr, Cd, Pb, Cu, Zn, and Ni were 39.1, 15.0, 32.8, 35.2, 785.4 and 49.3 kg km^{-2} yr^{-1}, respectively. When annual total fluxes are compared with similar measurement results obtained from previous studies (Table 2.3), all heavy metal fluxes in Izmir were higher by 1-2 orders of magnitude. Similar heavy metal fluxes for Pb and Cu in Belgrade, Yugoslavia were reported by Tasić et al. (2001).

Table 4.15 gives the correlation coefficients of dry deposition fluxes with the concentrations of soluble and total parts of the metals in the samples. Strong relationships have been observed between soluble and total concentrations and fluxes of Cr, Cd and Cu. Wu et al. (2006) have reported the relations between fluxes and total suspended, coarse and fine particulate concentrations of metals and found strong relations for Cr, Pb, Cu and Zn. Their results indicated that the flux was highly correlated with coarse particle concentrations for most of the elements because of the particles larger than 2.5 µm in size have high deposition velocities. Maneux et al. (1999) have reported that the dissolved fraction of heavy metals in rain was greater than the suspended fraction.

Table 4.15 Correlations between concentration and flux values of heavy metals in soluble and total forms in aqueous dry deposition samples

Sample Fraction		**Metal**					
		Cr	**Cd**	**Pb**	**Cu**	**Zn**	**Ni**
Soluble	r^2	***0.549***	***0.335***	0.146	***0.591***	0.243	0.286
	P	***<0.01***	***<0.05***	>0.05	***<0.01***	>0.05	>0.05
Total	r^2	***0.588***	***0.344***	0.174	***0.468***	0.117	0.181
	p	***<0.01***	***<0.05***	>0.05	***<0.01***	>0.05	>0.05

Table 4.16 shows the correlations between soluble and total concentration and flux values in wet deposition samples. Statistically strong correlations were found between soluble and total concentrations and fluxes for Cd, Pb, Cu and Zn. Luo (2001) has reported strong correlation coefficient between wet deposition fluxes with the concentrations for Cr. This observation indicated that the mechanism of the wet

deposition is controlled by different factors such as rain volume along with the concentration.

Table 4.16 Correlations between concentration and flux values of heavy metals in soluble and total in wet deposition samples

Sample Fraction		**Metal**					
		Cr	**Cd**	**Pb**	**Cu**	**Zn**	**Ni**
Soluble	r^2	0.123	***0.502***	***0.431***	***0.792***	***0.663***	0.004
	p	>0.05	***<0.01***	***<0.05***	***<0.01***	***<0.01***	>0.05
Total	r^2	0.084	***0.471***	***0.436***	***0.729***	***0.713***	0.000
	p	>0.05	***<0.01***	***<0.05***	***<0.01***	***<0.01***	>0.05

4.3 Toxicity Evaluation of Heavy Metals in Deposition

4.3.1 Inhibition Values

4.3.1.1 Inhibition Values of Simulated Single Heavy Metal Solutions

Time dependent changes on observed toxicity occur due to several processes that interact, including hydrolysis, photo-decomposition, membrane diffusion, and chemical reaction with the substrate (Kaiser & Palabrica, 1991). Keeping this in mind, the time of exposure of 15 minutes was selected for use in this study after preliminary tests for optimization. This is also in parallel with the literature information.

Bacterial responses in each new bacteria package were checked to confirm the dichromate reference point provided in the test kit. An average percent inhibition of 45% for the reference solution of 4 mg L^{-1} $K_2Cr_2O_7$ was found that checks well with the reference value of 42% given for the bacterial lot.

Toxicity evaluation criteria for luminescent bacteria indicates that the effect is “non-toxic” if the inhibitions are between 0% and 5%; “possibly toxic” if the inhibitions are between 5% to 20%, and "toxic" if the inhibitions are above 20% (Dr. Lange, 1994).

Table 4.17 shows the average percent inhibition values found for the luminescent bacteria for single heavy metal synthetic solutions. Low concentrations of heavy metals could make toxic effect. Mean concentrations of Cd and Ni were found possibly toxic. Mean concentrations of Cr, Pb, Cu and Zn have toxic effects and the higher concentrations of all heavy metals were found toxic except Ni according to this evaluation.

Table 4.17 Inhibitions of single heavy metals in aqueous solution at 15 min LUMIStox® test

Heavy Metals	**Selected Concentration in The Deposition Flux Range**	**Concentration (mg L^{-1})**	**Inhibition (%)**
Cr	Low	0.002	18.13±0.40
	Medium	0.005	23.25±1.08
	High	0.0125	29.35±0.43
Cd	Low	0.0025	11.64±0.34
	Medium	0.0075	17.60±0.81
	High	0.025	25.75±0.57
Pb	Low	0.001	7.06±1.29
	Medium	0.025	22.35±1.81
	High	0.1	40.67±1.83
Cu	Low	0.0025	10.02±0.17
	Medium	0.02	21.18±0.10
	High	0.04	24.46±0.25
Zn	Low	0.08	15.76±0.97
	Medium	0.2	23.92±1.69
	High	0.4	31.45±0.59
Ni	Low	0.001	7.26±0.52
	Medium	0.02	14.92±0.57
	High	0.05	17.01±0.74

4.3.1.2 Inhibition Values of Synthetic Metal Mixtures

Inhibition values of synthetic mixtures measured at the end of 15 min exposure time are given in Table 4.18. All mixed metal synthetic solutions were found toxic as the percentage inhibitions for 15 minutes were in the range 20-90%. Inhibition range of synthetic metal mixtures was 35.6 to 40.9%.

Table 4.18 Inhibition values of mixed metal synthetic solutions

Sample	**Heavy Metal Concentrations in Synthetic Mixture ($\mu g\ L^{-1}$)**						**Inhibition (%)**
	Cr	**Cd**	**Cu**	**Pb**	**Zn**	**Ni**	
Mixture 1	1000	2.5	2.5	1	80	1	40.9±0.6
Mixture 2	2	1000	2.5	1	80	1	39.7±1.0
Mixture 3	2	2.5	1000	1	80	1	37.7±0.4
Mixture 4	2	2.5	2.5	1000	80	1	38.9±0.5
Mixture 5	2	2.5	2.5	1	1000	1	36.5±0.4
Mixture 6	2	2.5	2.5	1	80	1000	35.6±0.5

4.3.1.3 Inhibition Values of Dry Deposition Samples

Inhibition values of dry deposition samples at the end of 15 min LUMIStox® test are given in Table 4.19. When Dr. Lange (1994) criteria were used for evaluating the average toxicity, all dry deposition samples were found to be in the "toxic" range. Inhibition values of WSS samples representing the dry deposition ranged from 22.3 to 58.3%.

The relationship between dissolved and total inhibition values of dry deposition samples is shown in Figure 4.14. There is significant statistical relationship between total and dissolved inhibition values of dry deposition samples at the 95% confidence level. Paired t-test was applied the dissolved and total inhibition values of dry deposition samples. It was demonstrated that there is no significant difference among these data sets. This indicates that the metals in the unfiltered samples did not create high-bias results due to physical absorption or scattering of light.

Table 4.19 Inhibition values of dry deposition samples

Sample	Inhibition Values for Total Concentration of Samples (%)	Inhibition Values for Dissolved Concentration of Samples (%)
DD-1	32.9±0.8	33.1±0.8
DD-2	28.8±0.8	30.2±0.6
DD-3	33.9±0.7	34.1±0.6
DD-4	33.7±0.9	33.8±0.4
DD-5	22.3±0.6	22.4±0.3
DD-6	37.3±4.2	38.3±5.2
DD-7	38.3±2.0	41.2±0.4
DD-8	37.7±0.8	37.6±1.0
DD-9	31.5±0.2	35.6±0.9
DD-10	33.0±0.4	30.5±0.9
DD-11	41.3±2.0	41.7±2.3
DD-12	54.2±8.4	58.3±5.9
Avg.inhibition±SD	**35.4±7.7**	**36.4±8.7**

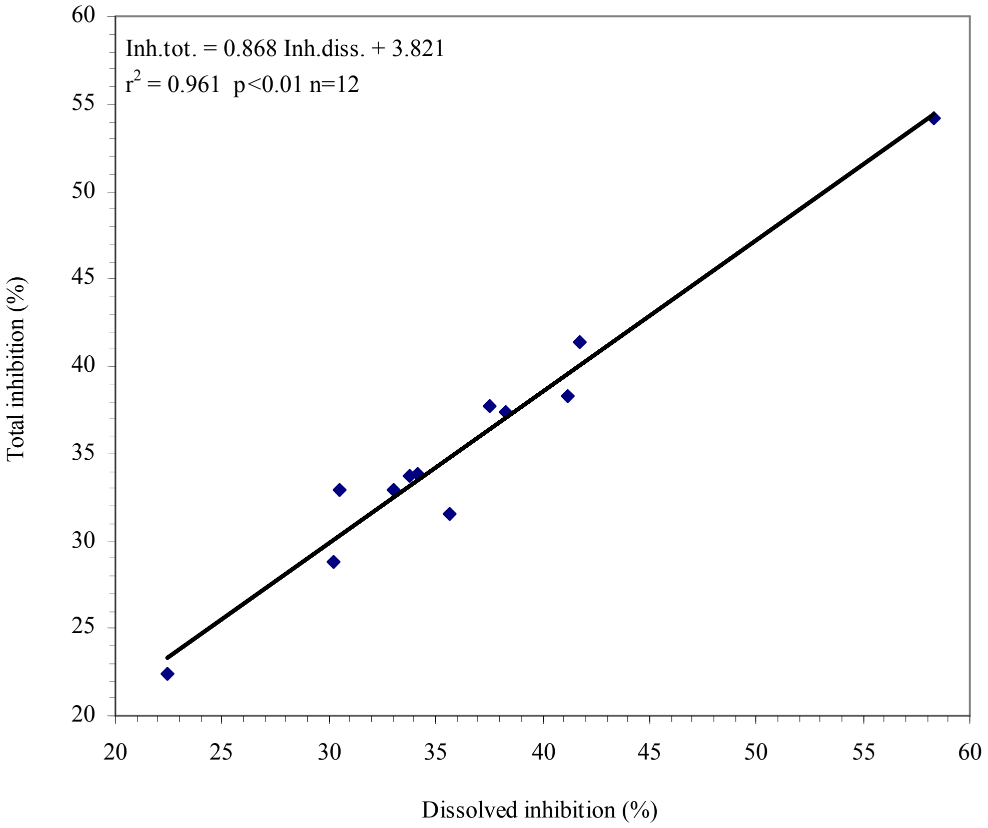


Figure 4.14 Relationship between dissolved and total inhibition values of dry deposition samples.

Statistical relationship between inhibition values of total and dissolved portions and pH values of dry deposition samples was investigated. Results of this statistical evaluation have shown that there is no relationship between inhibition values and pH values of samples because of the p value for the slope is greater than 0.05.

A multiple linear regression model to describe the relationship between inhibition values and dissolved and total concentrations of dry deposition samples are given below. The equation 4.1 of the fitted model for dissolved metal concentrations and inhibition values is shown as follow:

$$\text{Inhibition} = 53.23+1.84xC_{Cr}+0.14xC_{Cd}-0.09xC_{Pb}+0.07xC_{Cu}-0.08xC_{Zn}-1.99xC_{Ni} \qquad (4.1)$$

The equation 4.2 of the fitted model for total concentrations and inhibition values is given as:

$$\text{Inhibition} = 48.93+1.07xC_{Cr}-0.39xC_{Cd}+1.49xC_{Pb}+0.02xC_{Cu}-0.05xC_{Zn}-2.27xC_{Ni} \qquad (4.2)$$

Heavy metal concentrations found in the analyses were located on these inhibition-concentration equations for each dry deposition sample. The correlation coefficients of modeled and measured inhibition values of dissolved and total portions were 0.321 ($p<0.05$) and 0.331 ($p<0.05$), respectively (Figures 4.15 and 4.16). The statistical program suggests if Cd (for dissolved fraction) and Cu (for total fraction) are neglected, the results would not be affected. When these metals are neglected in another run as suggested.

According to the results of this second test run, the correlation coefficients of dissolved and total portions were nearly the same; 0.319 ($p<0.05$) and 0.334 ($p<0.05$) for soluble and total parts, respectively. In both cases the low p values shows that the toxicities and concentrations are somewhat interdependent. However, these models must be tested with more toxicity versus concentration data sets.

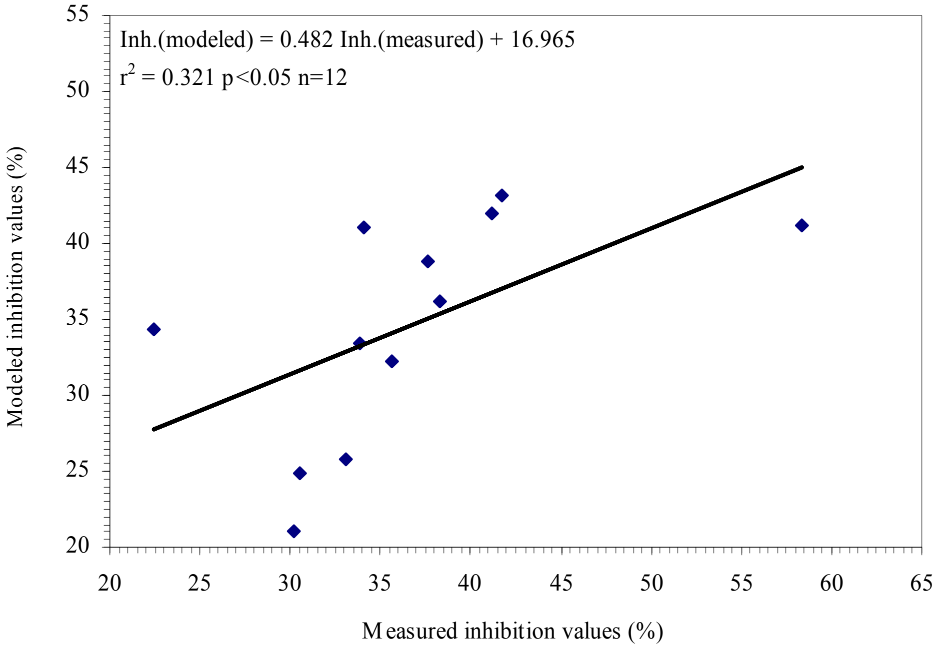


Figure 4.15 Relation between modeled and measured inhibition values for dissolved fraction of dry deposition samples.

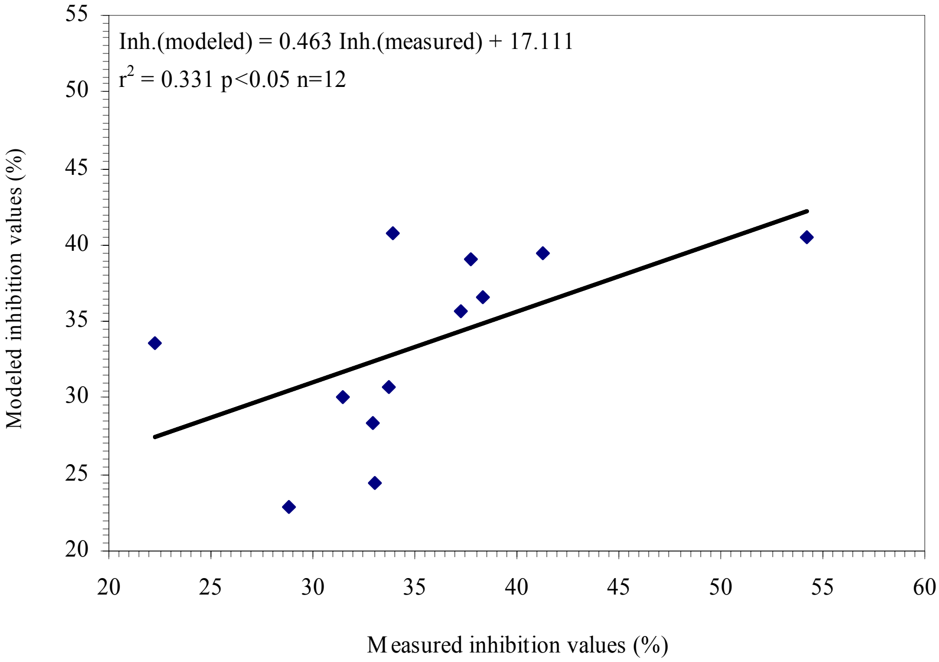


Figure 4.16 Relation between modeled and measured inhibition values for total fraction of dry deposition samples.

4.3.1.4 Inhibition Values of Rainwater Samples

Inhibition values of rainwater samples at the end of 15 min LUMIStox® test are given in Table 4.20. When Dr. Lange (1994) criterion is used for evaluating the average toxicity, all wet deposition samples were found to be in the "toxic" range. Inhibition values of rainwater samples ranged from 20.4-38.0%.

Table 4.20 Inhibition values of rainwater samples

Sample	**Inhibition Values for Total Concentration of Samples (%)**	**Inhibition Values for Dissolved Concentration of Samples (%)**
WD-1	36.8±1.5	38.0±0.7
WD-2	26.8±0.6	27.8±0.9
WD-3	20.6±1.3	20.5±1.6
WD-4	23.2±1.1	25.2±1.2
WD-5	23.6±0.7	23.4±0.6
WD-6	26.5±2.0	33.0±1.0
WD-7	20.4±1.7	21.5±1.8
WD-8	28.4±2.1	31.0±2.3
WD-9	33.1±3.5	30.9±1.2
WD-10	28.4±1.2	29.9±3.2
WD-11	30.7±0.6	32.5±1.1
WD-12	35.2±1.4	36.1±2.1
WD-13	30.0±1.6	32.9±1.8
Avg.inhibition±SD	**28.0±5.2**	**29.4±5.4**

The relationship between inhibition values of dissolved and total metals of rainwater samples is shown in Figure 4.17. There was a significant statistical correlation between total and dissolved inhibition values of wet deposition samples at the 95% confidence level. Paired t-test was applied the dissolved and total inhibition values of wet deposition samples. It was demonstrated that there is no significant difference among these data sets.

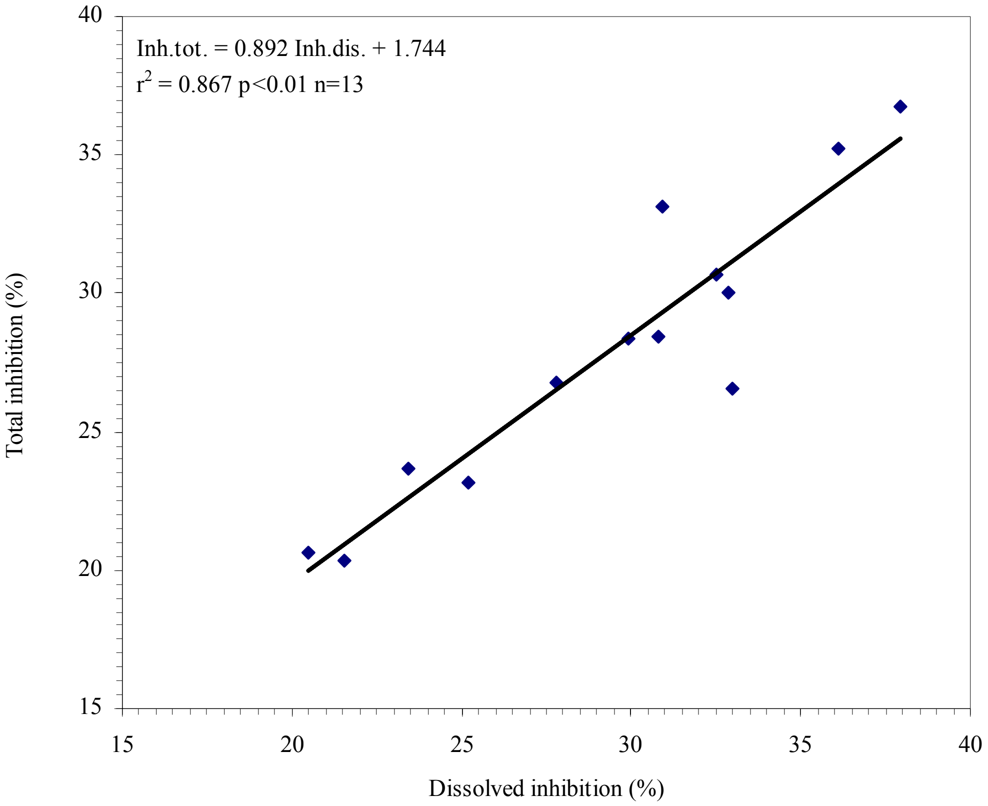


Figure 4.17 Relation between dissolved and total inhibition values of wet deposition samples.

Statistical relationship between inhibition values of total and dissolved parts of the metals and pH values of wet deposition samples was also investigated. Results of this evaluation have shown that there is no relationship between inhibition values and pH values of samples because of the p value for the slope is greater than 0.05.

The relationship between dissolved and total concentrations of wet deposition samples and inhibition values were separately investigated, too. The equation 4.3 of the fitted model for dissolved rainwater concentration and inhibition values is shown as follow:

$$\text{Inhibition} = -9.93+0.73\text{x}C_{Cr}-2.73\text{x}C_{Cd}-0.08\text{x}C_{Pb}+0.28\text{x}C_{Cu}-0.07\text{x}C_{Zn}+6.87\text{x}C_{Ni} \qquad (4.3)$$

The equation 4.4 of the fitted model for total concentration and inhibition values is given below:

$$\text{Inhibition} = 12.31+0.08xC_{Cr}+0.14xC_{Cd}-0.66xC_{Pb}+0.09xC_{Cu}-0.03xC_{Zn}+2.89xC_{Ni} \qquad (4.4)$$

Heavy metal concentrations found in the analyses were located on these inhibition-concentration equations for each wet deposition sample. The correlation coefficients of modeled and measured inhibition values of dissolved and total portions were 0.331 ($p<0.05$) and 0.444 ($p<0.05$), respectively (Figures 4.18 and 4.19). The statistical program suggests if Pb (for dissolved fraction) and Cd (for total fraction) are neglected, the results would not be affected. When these metals are neglected in another run as suggested.

According to the results of this second test run, the correlation coefficients of dissolved and total portions were nearly the same; 0.331 ($p<0.05$) and 0.455 ($p<0.05$) for soluble and total parts, respectively. In both cases the low p values shows that the toxicities and concentrations are somewhat interdependent. However, these models must be tested with more toxicity versus concentration data sets.

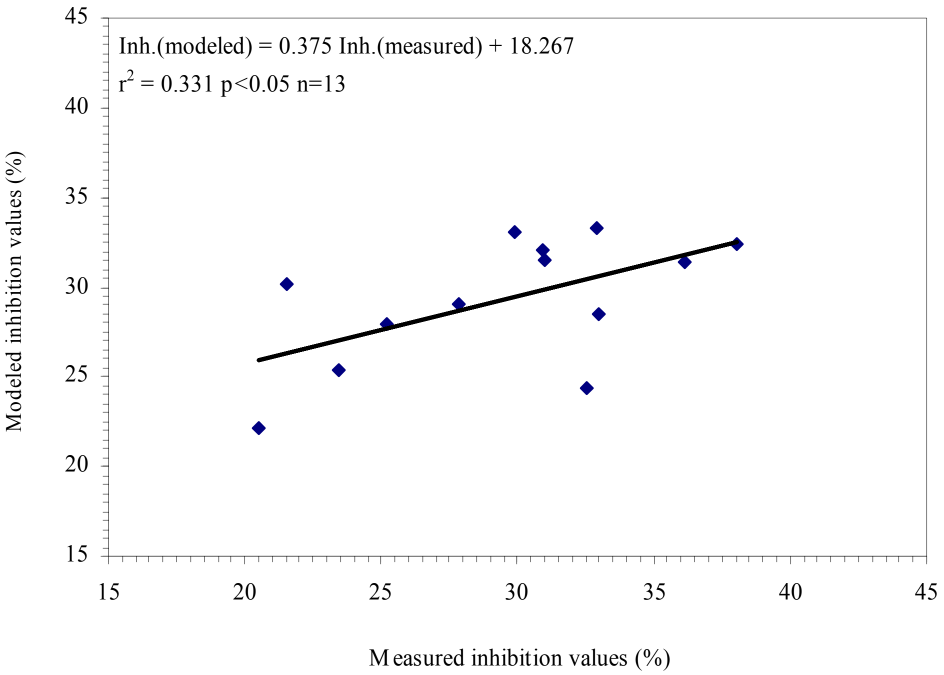


Figure 4.18 Relationship between modeled and measured inhibition values for dissolved fraction of wet deposition samples.

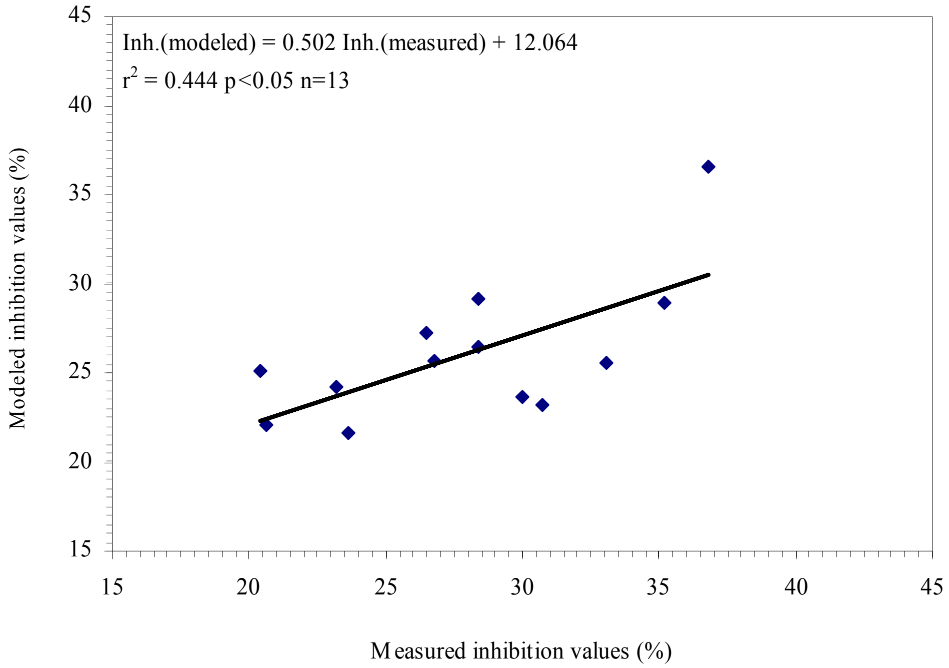


Figure 4.19 Relationship between modeled and measured inhibition values for total fraction of wet deposition samples.

4.3.2 Effective Concentrations of Deposited Heavy Metals in Dry and Wet Forms on the Toxicity of Receiving Waters

4.3.2.1 EC_{50} Values of Simulated Single Heavy Metal Solutions

Table 4.21 shows the EC_{50} values that were calculated from two different formulas suggested by Mowat & Bundy (2002a) and Jennings et al. (2001). Details of these formulas were given in Section 3.5.3.

A statistical evaluation was made to understand if there is significant difference between EC_{50} values calculated from Formula 1 and Formula 2 that were suggested by Mowat & Bundy (2002a) and Jennings et al. (2001), respectively. According to the results of this statistical evaluation, EC_{50} values calculated each formula were the same (Figure 4.20). In addition, paired t-test was applied the EC_{50} values calculated from Formula 1 and Formula 2. It was demonstrated that there is no significant difference among these data sets.

Table 4.21 Concentrations of the single metal solutions and EC_{50} values

Metal	Concentration Range (µg L^{-1})			EC_{50} Values (µg L^{-1})	
	Lower	**Middle**	**Upper**	**Formula 1**[*]	**Formula 2**[**]
Cr	2.0	5.0	12.5	161±19	161±19
Cd	2.5	7.5	25.0	305±29	305±29
Pb	1.0	25.0	100.0	413±17	413±19
Cu	2.5	20.0	40.0	621±13	634±11
Zn	80.0	200.0	400.0	1567±54	1616±98
Ni	1.0	20.0	50.0	22660±1855	24294±2007

[*] : Formula 1 was suggested by Mowat & Bundy (2002a).

[**]: Formula 2 was suggested by Jennings et al. (2001).

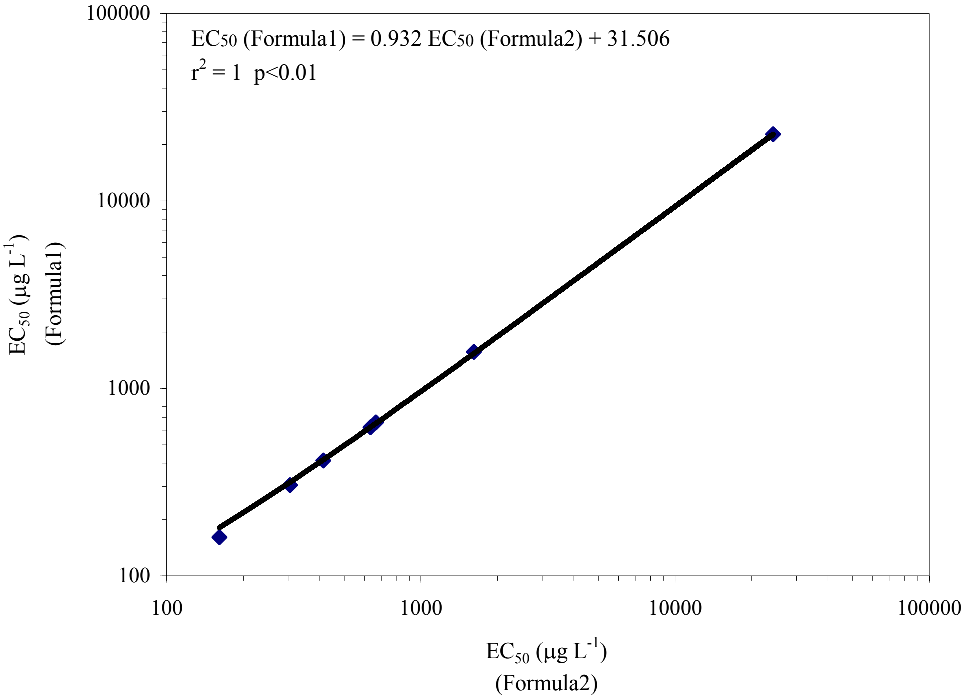


Figure 4.20 Comparison of EC_{50} values calculated from two different formulas.

LUMIStox® toxicity tests were repeated for 6 times for Cr and Cd, and 5 times for other metals. From the Table 4.21 it may be noted that Cr had the lowest EC_{50} (therefore most toxic) and Ni has the highest EC_{50} (therefore least toxic) of the six heavy metals. Toxicity ranking of metals from most to least toxic was found as Cr>Cd>Pb>Cu>Zn>Ni. In this study no other citation could be found in the literature with LUMIStox® test method applied to the deposited heavy metal toxicity at the levels induced by airborne heavy metals. All of the toxicity evaluations cited in the literature were made according to the heavy metal concentration levels at polluted waters and sediments by using Microtox® test. Table 2.6 summarizes the cited EC_{50} values and studied ranges of concentrations in the literature. The EC_{50} values of synthetic single heavy metal solutions determined in this study is found comparable with the data in the literature for comparable levels in solution. When metals were listed from most to least toxic according to the EC_{50} values in Table 2.6, toxicity ranking of Pb>Cu>Zn>Ni>Cd>Cr was obtained. If the concentrations causing 50% inhibition in bacteria found from lower (sub-ppm) concentration ranges in this study are used, relative toxicity ranking of metals are not as shown in the list given Table 2.6. This relative toxicity list seems rather peculiar especially for the place of the last two metals. This is in contrast to the fact that Cd and Cr are among the most toxic heavy metal pollutants. The list we obtained seems more understandable considering the ecological properties of these metals.

EC_{50} value found for Cr in this study is slightly lower than the cited values in the literature which refers to higher toxicity.

Results obtained by other researchers who used solutions with high Cd concentrations for the Microtox® test point to low toxic effects to the bacteria. As this was unexpected by these researchers, too, they have concluded that Microtox® is not as sensitive to cadmium as are other microbial tests such as *Pseudomonas fluorescens* (Codina et al., 1998). The reduction in cadmium toxicity was explained, in part, by the presence of exopolysaccharides (EPS) on the outer layer of gram-negative bacteria which have been found to adsorb cadmium at a protective zone around the cells. Cadmium is thus stored at high concentrations in the protective hydrophobic space between the inner and outer walls of the bacteria, where it cannot exert its toxicity. This renders gram-negative bacteria more resistant to cadmium in the environmental medium, as the sites within the cell will be saturated and further adsorption is prevented (Mowat & Bundy, 2002a). In contrast, Sillanpaa & Oikari (1996) have reported 300 $\mu g\ L^{-1}$ EC_{50} value for Cd in low concentrations as similar our result.

Good agreement was found for lead, copper and zinc to estimate the toxicities with the literature similar to the values cited by Ince et al. (1999). In addition a smaller EC_{50} value for Zn was found by Sillanpaa & Oikari (1996). EC_{50} for Ni in this study is within the range of the literature data.

Eh and pH are two important parameters in determining the ionic species of metals in solution as ionic species are known to exhibit different toxicities on *Photobacterium phosphoreum* populations. At high concentration solutions, presence of excessive amounts of chlorides added as salt for acclimation of bacteria might cause critical precipitation effects. This becomes especially important as the metal concentrations approach or exceeds the solubility products of metal chlorides. Special care must be exercised in testing lead as its chlorides have lower solubility products than the others. But in this study the presence of extremely low concentration metals at ppb ranges have not been subject to such errors.

4.3.2.2 $EC_{50total}$ Values of Synthetic Metal Mixtures

$EC_{50total}$ values calculated for mixed metal synthetic solutions are given in Table 4.22. From this table it may be seen that Mixture 1 is the most toxic and Mixture 6 is the least toxic of the six heavy metal mixtures. In Table 4.22 C_1 indicates the parameter in model proposed in equation 3.10 to 3.17. Whichever metal is used as C_1 in this calculation the resulting $EC_{50total}$ is the same with a variation of around 10%. $EC_{50total}$ values showing the toxicities ranged between around 473.2 and 903.3 µg L^{-1} in synthetic mixtures 1-6. In this order Mixture 6 with the leading compound of Ni has the lowest and Mixture 1 with the leading compound of Cr has the highest toxicity. These figures indicate much less toxicity in mixture compared to the single metal solutions separately evaluated for toxicity. This may be due to the antagonism among the heavy metals as discussed in more detail in 4.3.3.4 section. The determination of $EC_{50total}$ values of mixed metal synthetic solutions has allowed evaluating the interactive effect of heavy metals in dry and wet deposition samples.

Table 4.22 Metal concentrations in the mixed metal solutions and their calculated $EC_{50total}$ values with C_1 concentrations in each calculation run

Sample	**$EC_{50total}$ Values for Leading Metal ($\mu g\ L^{-1}$)**						**$EC_{50total}$ Values ($\mu g\ L^{-1}$)**
	C_1:Cr	**C_1:Cd**	**C_1:Cu**	**C_1:Pb**	**C_1:Zn**	**C_1:Ni**	
Mixture 1	469.6	482.9	469.1	467.3	476.6	473.7	473.2±5.8
Mixture 2	499.3	499.8	499.5	513.4	507.6	504.4	504.0±5.7
Mixture 3	570.0	555.1	554.8	552.6	563.7	560.2	559.4±6.6
Mixture 4	499.9	500.4	514.7	499.0	509.0	505.8	504.8±6.2
Mixture 5	705.2	706.0	705.6	702.9	725.2	712.5	709.6±8.3
Mixture 6	896.8	897.7	897.3	893.8	911.7	922.2	903.3±11.2

4.3.2.3 $EC_{50total}$ Values of Dry Deposition Samples

$EC_{50total}$ values of dry deposition samples are shown in Table 4.23. From this table it may be seen that sixth and ninth DD sample was the most toxic and second DD sample was the least toxic. When $EC_{50total}$ values were compared, all dry deposition samples were much more toxic than all of the synthetic mixtures of similar heavy metal concentrations possibly due to the additive effects of other air pollutant depositions. $EC_{50total}$ values ranged between around 74.3 and 221.0 $\mu g\ L^{-1}$ in dry deposition samples. The mean value of $EC_{50total}$ was 134.1±39.8 $\mu g\ L^{-1}$ for these samples.

Table 4.23 includes six different $EC_{50total}$ values of dry deposition samples between columns 2-7. These values have been computed from six different runs each with a different C_1 compound using the equation 3.10 to 3.17. The results are the same within the standard deviation shown in the last column giving the average $EC_{50total}$ value of each different run. No statistically significant relationships were found between $EC_{50total}$ values and dissolved concentrations of heavy metals in dry deposition samples except for dissolved Zn ($r^2=0.693$; $p<0.01$).

Table 4.23 $EC_{50total}$ values of dry deposition samples

Sample	**$EC_{50total}$ Values for Leading Metal (µg L^{-1})**						**$EC_{50total}$ Values (µg L^{-1})**
	C_1:Cr	**C_1:Cd**	**C_1:Cu**	**C_1:Pb**	**C_1:Zn**	**C_1:Ni**	
DD-1	140.4	140.3	140.2	140.8	142.8	141.2	141.0±1.0
DD-2	220.7	219.5	219.9	220.3	224.5	220.9	221.0±1.8
DD-3	118.7	117.6	118.1	118.3	120.0	118.9	118.6±0.8
DD-4	168.7	167.4	168.0	168.4	171.2	169.1	168.8±1.3
DD-5	148.8	148.5	149.7	149.1	151.6	149.1	149.5±1.1
DD-6	75.8	75.3	75.7	75.8	76.5	76.0	75.9±0.4
DD-7	131.9	131.1	131.4	131.4	133.6	131.6	131.8±0.9
DD-8	153.1	153.1	153.4	153.5	156.0	153.7	153.8±1.1
DD-9	74.2	73.8	74.3	74.3	75.0	74.4	74.3±0.4
DD-10	124.4	124.5	124.7	124.8	126.6	125.3	125.1±0.8
DD-11	140.2	138.6	139.2	140.0	141.8	140.1	140.0±1.1
DD-12	108.2	108.3	108.9	108.6	110.1	108.7	108.8±0.7
Average	**133.8**	**133.2**	**133.6**	**133.8**	**135.8**	**134.1**	**134.1±39.8**
SD	**39.7**	**39.5**	**39.5**	**39.6**	**40.5**	**39.7**	

Simple regression analysis was applied to determine the relationship between pH and $EC_{50total}$ values. There was no statistically significant relationship between $EC_{50total}$ and pH values of dry deposition samples as the p value was found above 0.05. Similarly, no relationship was found between $EC_{50total}$ and inhibition values of dissolved portions of dry deposition samples as the p values were above 0.05.

4.3.2.4 $EC_{50total}$ Values of Rainwater Samples

$EC_{50total}$ values of wet deposition samples are shown in Table 4.24. From this table it may be seen that twelfth WD sample was the most toxic and second WD sample was the least toxic. When $EC_{50total}$ values were compared, all wet deposition samples were more toxic than all of the synthetic mixtures as was the case with the dry deposition samples. $EC_{50total}$ values ranged from 71.9 to 224.7 µg L^{-1} in wet deposition samples. The mean value of $EC_{50total}$ was 106.0±44.2 µg L^{-1} for the rainwater samples.

In computing the EC$_{50total}$ values of wet deposition samples, six different runs were made each with a different C$_1$ compound according to the method described above. The results are the same within the standard deviation shown in the last column of Table 4.24 giving the average EC$_{50total}$ value of each different run.

Table 4.24 EC$_{50total}$ values of rainwater samples

Sample	**EC$_{50total}$ Values for Leading Metal (μg L^{-1})**						**EC$_{50total}$ Values (μg L^{-1})**
	C$_1$:Cr	**C$_1$:Cd**	**C$_1$:Cu**	**C$_1$:Pb**	**C$_1$:Zn**	**C$_1$:Ni**	
WD-1	96.2	95.3	96.4	95.8	97.1	95.6	96.1±0.6
WD-2	223.6	222.6	225.5	224.0	228.4	224.0	224.7±2.0
WD-3	127.3	125.7	126.3	126.2	128.3	126.7	126.8±0.9
WD-4	93.0	92.1	93.0	92.9	94.0	93.0	93.0±0.6
WD-5	80.8	80.6	81.0	81.1	81.9	80.9	81.1±0.5
WD-6	75.4	75.0	75.2	75.0	76.0	75.1	75.3±0.4
WD-7	92.0	91.1	90.9	91.2	92.4	91.3	91.5±0.6
WD-8	83.7	83.0	83.0	83.2	84.2	83.3	83.4±0.5
WD-9	90.2	89.3	89.4	89.6	90.7	89.7	89.8±0.5
WD-10	72.4	72.1	73.0	72.3	73.0	72.2	72.5±0.4
WD-11	103.8	102.6	103.1	103.3	104.6	103.5	103.5±0.7
WD-12	71.8	71.5	72.0	71.7	72.5	71.8	71.9±0.3
WD-13	168.1	166.7	168.3	167.4	170.6	167.7	168.1±1.3
Average	**106.0**	**105.2**	**105.9**	**105.7**	**107.2**	**105.8**	**106.0±44.2**
SD	**44.0**	**43.7**	**44.4**	**44.0**	**45.1**	**44.1**	

On the average, rainwater samples were found 15% more toxic than dry deposition samples, although the ranges of toxicities were nearly the same. This shows that the rainwater samples representing the wet deposition were slightly more toxic to bacteria. Winter rain was more effective on toxicity. Statistically significant relationship was found between EC$_{50total}$ values of rainwater samples and air temperature values on sampling days ($r^2=0.431$; $p<0.05$).

When statistical relations are investigated between EC$_{50total}$ values and concentrations, no statistically significant relationships were found between EC$_{50total}$

values and dissolved concentrations of heavy metals in wet deposition samples except Zn ($r^2=0.862$; $p<0.01$), and Ni ($r^2=0.417$; $p<0.05$).

Simple regression analysis was applied to determine the relationship between pH values and $EC_{50total}$ values. There was no statistically significant relationship between $EC_{50total}$ and pH values of wet deposition samples ($r^2=0.106$; $p>0.05$). Similarly, no relationship was found between $EC_{50total}$ and inhibition values of dissolved portions of wet deposition samples as the p values were above 0.05.

Water concentrations of heavy metals generated by airborne heavy metals over relatively short periods of time such as one day are lower than the levels in polluted waters or sediments which were investigated by many researchers who studied the toxicities of metals. It is already shown that the ionic strengths of individual metal solutions have a notable impact on the toxicity test results based on *Vibrio fischeri* such as cited by Ownby (2002). But in our case of low concentrations of heavy metals due to air pollutant depositions, samples were relatively free from heavy ionic strength cross-impacts and dependable $EC_{50total}$ values were obtained.

4.3.3 Toxicity Index

For toxicity evaluation of synthetic mixtures, dry and wet deposition samples, Toxicity Index (TI) values described in Section 3.5.5 were determined. This index should be evaluated as the component with the highest TI value having the highest impact on overall toxicity in the mixtures (Mowat & Bundy, 2002b).

4.3.3.1 Toxicity Index Values of Heavy Metals in Synthetic Metal Mixtures

TI values of synthetic mixed solutions are shown in Table 4.25. For each solution the highest TI value is underlined to show the leading toxic metal. The most toxic component for overall toxicity is underlined. From this Table it may be seen that TI values of chromium, cadmium, copper, lead and zinc indicate a high potential contribution to the overall toxicities of Mixture 1, Mixture 2, Mixture 3, Mixture 4 and Mixture 5, respectively. However, zinc is leading toxicant in Mixture 6 although Ni was the leading heavy metal in concentration.

Table 4.25 TI values of heavy metals in simulated mixed metal synthetic solutions

Sample	**TI Values**					
	Cr	**Cd**	**Cu**	**Pb**	**Zn**	**Ni**
Mixture 1	**6.211**	0.008	0.004	0.002	0.051	0.001
Mixture 2	0.012	**3.279**	0.004	0.002	0.051	0.001
Mixture 3	0.012	0.008	**1.610**	0.002	0.051	0.001
Mixture 4	0.012	0.008	0.004	**2.421**	0.051	0.001
Mixture 5	0.012	0.008	0.004	0.002	**0.638**	0.001
Mixture 6	0.012	0.008	0.004	0.002	**0.051**	0.044

4.3.3.2 Toxicity Index Values of Heavy Metals in Dry Deposition Samples

TI values of dry deposition samples are indicated in Table 4.26. From this table it is seen that toxic effect of chromium is leading in the DD-6 and DD-9 samples. Toxic effect of zinc is leading in all of the other DD samples.

4.3.3.3 Toxicity Index Values of Heavy Metals in Rainwater Samples

TI values for each metal in wet deposition samples are shown in Table 4.27. According to this table toxic effect of zinc is leading in the WD-2, WD-5, WD-12 and WD-13 samples whilst the toxic effect of copper is leading in the WD-10. Toxic effect of chromium is leading in rest of the rainwater samples.

Results showed that the studied metals contributed to the toxicity of environmental samples. Especially Zn (not necessarily the most toxic but always at high concentrations) and Cr (the most toxic) contributed to toxicity in most of the samples.

Table 4.26 TI for each metal in dry deposition samples

Sample	**TI Values of Heavy Metals**					
	Cr	**Cd**	**Pb**	**Cu**	**Zn**	**Ni**
DD-1	0.018	0.009	0.016	0.004	**0.092**	0.0005
DD-2	0.044	0.007	0.013	0.006	**0.217**	0.0004
DD-3	0.044	0.003	0.008	0.004	**0.049**	0.0004
DD-4	0.033	0.003	0.009	0.004	**0.091**	0.0004
DD-5	0.022	0.007	0.011	0.022	**0.126**	0.0002
DD-6	**0.067**	0.007	0.022	0.011	0.044	0.0007
DD-7	0.070	0.011	0.013	0.009	**0.129**	0.0004
DD-8	0.011	0.006	0.008	0.005	**0.071**	0.0002
DD-9	**0.050**	0.007	0.025	0.019	**0.050**	0.0007
DD-10	0.013	0.009	0.011	0.006	**0.072**	0.0005
DD-11	0.057	0.002	0.017	0.003	**0.074**	0.0004
DD-12	0.011	0.007	0.009	0.011	**0.052**	0.0002

Table 4.27 TI for each metal in rainwater samples

Sample	**TI Values of Heavy Metals**					
	Cr	**Cd**	**Pb**	**Cu**	**Zn**	**Ni**
WD-1	**0.158**	0.010	0.022	0.057	0.119	0.0002
WD-2	0.041	0.008	0.020	0.063	**0.399**	0.0004
WD-3	**0.081**	0.002	0.004	0.003	0.044	0.0002
WD-4	**0.036**	0.002	0.012	0.008	0.034	0.0002
WD-5	0.030	0.009	0.029	0.016	**0.054**	0.0003
WD-6	**0.110**	0.015	0.011	0.013	0.053	0.0003
WD-7	**0.151**	0.011	0.011	0.003	0.047	0.0002
WD-8	**0.099**	0.008	0.010	0.005	0.037	0.0003
WD-9	**0.112**	0.007	0.010	0.004	0.042	0.0002
WD-10	0.076	0.017	0.018	**0.108**	0.046	0.0002
WD-11	**0.066**	0.003	0.009	0.004	0.038	0.0002
WD-12	0.039	0.010	0.013	0.021	**0.041**	0.0003
WD-13	0.090	0.008	0.013	0.033	**0.242**	0.0004

Toxicity index data shows that the studied metals account for only part of the overall toxicity of the samples. The reason of this may be the interactions between heavy metals in the form of antagonism which arose due to the co-existence of the metals and other toxic ingredients in the solution created by wet deposition from the air.

4.3.3.4 Interactive Toxicity Effects of Metal Mixtures

Observed and calculated EC_{50M} values for a mixture of multiple toxicants are presented in Table 4.28. Observed EC_{50M} values are ranged between around 473.2 and 903.3 µg L^{-1} in synthetic mixtures 1 to 6, while calculated EC_{50M} values are ranged between 6.0 and 312.5 µg L^{-1} in synthetic mixtures. While calculated EC_{50M} values of mixtures suggest much high toxicity, observed EC_{50M} values were showed low toxicity. The antagonistic effect among the heavy metals was observed when observed EC_{50M} values were exceeding the calculated EC_{50M} values. For that reason LUMIStox® data provided antagonistic interactions for each heavy metal in this study.

Table 4.28 Observed and calculated EC_{50M} values for a mixture of multiple toxicants

Sample	EC_{50M} Values (µg L^{-1})		Classification of Toxicity Mixtures
	Observed	Calculated	
Mixture 1	473.2	7.1	Antagonistic effect
Mixture 2	504.0	7.1	Antagonistic effect
Mixture 3	559.4	6.0	Antagonistic effect
Mixture 4	504.8	22.1	Antagonistic effect
Mixture 5	709.6	50.4	Antagonistic effect
Mixture 6	903.3	312.5	Antagonistic effect

CHAPTER FIVE
CONCLUSIONS

5.1 Conclusions

Dry and wet deposition samples were collected between October 2003 and June 2004 in Buca, Izmir. Dry and wet depositions of selected heavy metals (Cr, Cd, Pb, Cu, Zn and Ni) were sampled using a water surface sampler. Heavy metals were analyzed by a Perkin-Elmer Model 700 atomic absorption spectrophotometer equipped with a graphite furnace (GFAAS) except Zn. For Zn analysis of samples flame-AAS was used in the same instrument to avoid erratic results in the graphite furnace due to high concentrations in the samples. Dry deposition samples were collected over 24 hours and wet deposition samples were taken over the rainy period.

In addition LUMIStox® toxicity test was used to determine the overall toxicity of the collected deposition samples. Overall toxicity levels in the environmental samples were compared with the individual toxicities due to the selected metals.

5.1.1 Concentrations of Aqueous Solutions Due To Wet Deposition

The average total heavy metal concentrations of Cr, Cd, Pb, Cu, Zn and Ni were found as 17.1±8.5, 3.1±1.6, 6.6±4.1, 19.5±24.8, 184.2±224.0, and 6.7±2.6 in $\mu g\ L^{-1}$, respectively for wet deposition samples. These concentrations were generally higher than the values previously measured at different sites around the world.

Among heavy metals in wet deposition samples, Zn had the highest level and Cd the lowest. The relative abundance of Zn was 70% for rainwater samples.

There are significant correlations between Cd-Cu and Zn-Ni concentrations in dissolved form, and Pb-Cu, Pb-Zn, Pb-Ni, and Zn-Ni concentrations in total form rainwater concentrations at the 95% confidence level. Strong relationships between these heavy metals indicate that they undergo similar reactions in the atmosphere before they were brought down by the raindrops.

Statistically significant relationships were found between air temperatures with Pb, Zn and Ni total concentrations at the 95% confidence level. The total rainwater concentrations of Pb, Zn, and Ni and wind speeds have also correlated well at the 95% confidence level. Dissolved and total concentrations of rainwater samples and the relative humidity values at the 95% confidence level did not correlate.

Dissolved and total heavy metal concentrations in wet deposition samples were not statistically correlated with the rain volumes on sampling day.

The ratio of soluble fractions of heavy metals in the wet deposition is within 37-100 percent range of the total metals in deposition. The ratio of soluble fractions of heavy metals in the dry deposition is within 44-100 percent range of the total metals in deposition.

5.1.2 Dry Deposition Fluxes

The measured average total dry deposition fluxes for Cr, Cd, Pb, Cu, Zn, and Ni were 96.4±69.2, 41.3±20.0, 90.3±40.8, 81.3±48.6, 2127.2±651.4, and 139.6±62.0 $\mu g\ m^{-2}\ day^{-1}$, respectively. The average dry deposition of Zn was the highest and the average Cd flux was the lowest of the metal fluxes. The dry deposition fluxes were appreciably higher in Izmir compared to elsewhere.

Highest correlations were found between the Pb and Ni soluble and total dry deposition flux fractions, respectively. This indicates that these metals have a common source, possibly of traffic origin at the study site.

When we relate the dry deposition heavy metal fluxes with corresponding wind directions, it is notable that higher depositions occurred when it blew from the north. On the contrary lower fluxes occurred with southerly winds.

5.1.3 Wet Deposition Fluxes

The measured average total wet deposition fluxes for Cr, Cd, Pb, Cu, Zn, and Ni were 271.2±148.1, 54.3±46.1, 111.5±77.9, 362.5±670.5, 2387.7±2807.2, and 107.8±49.7 µg m^{-2} day^{-1}, respectively. Zn has highest daily wet deposition fluxes; Cd has lowest daily wet deposition fluxes in this study.

The wet deposition fluxes of different metals are interrelated except with Zn. In soluble and total form, most important relationship was found between Cd and Cu.

When we compare wet deposition heavy metal fluxes with corresponding wind directions, higher values have occurred when it blew from the north mostly. On the contrary lower fluxes were found during southerly winds.

There were statistically significant relationships between wind speed values and dissolved and total fluxes of Zn ($r^2=0.779$; $p<0.01$ and $r^2=0.876$; $p<0.01$, respectively). The statistical relationships between air temperatures on sampling days and dissolved and total fluxes of Cr have been found to be $r^2=0.379$ ($p<0.05$) and $r^2=0.358$ ($p<0.05$), respectively. There was no statistically significant relationship between dissolved and total fluxes of heavy metals in wet deposition samples and relative humidity values at the 95% confidence level.

In this study, statistical relationships between daily rain volumes and daily soluble metal fluxes have been found to be $r^2=0.446$ ($p<0.05$) for Cr; $r^2=0.477$ ($p<0.01$) for Cd; $r^2=0.476$ ($p<0.01$) for Pb, and $r^2=0.875$ ($p<0.01$) for Ni. Daily rain volumes and daily total fluxes of Cr ($r^2=0.504$; $p<0.01$), Cd ($r^2=0.490$; $p<0.01$), Pb ($r^2=0.306$; $p<0.05$), and Ni ($r^2=0.745$; $p<0.01$) were correlated, too.

In Izmir, the relationships between daily rain period and daily dissolved and total fluxes of heavy metals have not been found.

5.1.4 Comparison of Dry and Wet Deposition Fluxes

The dry and wet deposition fluxes were appreciably higher in Izmir compared to elsewhere. The daily wet deposition fluxes were larger than the dry deposition fluxes by 2.8 times for Cr, 1.3 times for Cd, 1.2 times for Pb, 4.5 times for Cu, 1.1 times for Zn and 0.8 times for Ni. Wet deposition rates except Ni are more significant than the dry deposition rates which may on a daily basis.

The annual dry deposition fluxes of Cr, Cd, Pb, Cu, Zn and Ni were calculated as 32.2±23.1, 13.8±6.7, 30.1±13.6, 27.2±16.2, 710.5±217.6, and 46.6±21.3 kg km^{-2} yr^{-1}, respectively. Cr, Cd, Pb, Cu, Zn and Ni annual wet deposition fluxes were 6.9±3.5, 1.2±0.6, 2.7±1.7, 8.0±10.1, 74.9±91.1, and 2.7±1.1 kg km^{-2} yr^{-1}, respectively. The ratios of dry versus wet deposition per year were 4.7 times for Cr, 11.5 times for Cd, 11.1 times for Pb, 3.4 times for Cu, 9.5 times for Zn, and 17.3 times for Ni. Thus, dry deposition was more important than that of wet deposition throughout the study period in this suburban area.

Annual total (wet+dry) fluxes of Cr, Cd, Pb, Cu, Zn, and Ni was 39.1, 15.0, 32.8, 35.2, 785.4, and 49.3 kg km^{-2} yr^{-1}, respectively. When annual total fluxes are compared with similar measurement results obtained from previous studies, all heavy metal fluxes in Izmir were higher by 1-2 orders of magnitude.

5.1.5 Toxicity Evaluations

Toxicity evaluations are based on soluble fractions of the studied heavy metals. Toxicity ranking of metals from most toxic to least toxic in this study was found as Cr>Cd>Pb>Cu>Zn>Ni. Good agreement was found for studied heavy metals between our results and most of the reported work of others except chromium toxicity level. EC_{50} of chromium is slightly lower than those found in the literature. Along with the determinations of EC_{50}'s found from low heavy metal concentration ranges as in wastewater and leachate studies covered in the literature, the same tests were also applied to solutions having concentrations in the air pollutant deposition ranges. As no LUMIStox® application could be found in the literature for heavy metal toxicity of air pollution origin. For that reason results obtained in this study were compared with results for Microtox® test. We believe that the difference between these values may be originated from this lack of data. Also it must be kept in mind that the airborne heavy metal levels is much lower than the levels which are used by at the other researchers who have studied polluted waters or sediments mostly.

$EC_{50total}$ values ranged between around 74.3 and 221.0 µg L^{-1} in dry deposition samples. The mean value of $EC_{50total}$ was 134.1±39.8 µg L^{-1} for these samples. $EC_{50total}$ values ranged from 71.9 to 224.7 µg L^{-1} in wet deposition samples. The mean value of $EC_{50total}$ was 106.0±44.2 µg L^{-1} for the rainwater samples. Zinc was lead in dry deposition samples, while chromium was lead most of the rainwater samples. Especially Zn (not necessarily the most toxic but in high concentrations) and Cr (the most toxic) contributed to most of the toxicity.

Rainwater samples were found 15% more toxic than dry deposition samples, although the ranges of toxicity were nearly the same. The results of this study underline the importance of metal pollution in the rainwater especially in regard to the ecotoxic properties of heavy metals rather than the dry deposition which occurs on the long run.

Environmental samples are more toxic than synthetic mixed solutions. Here it must be emphasized that possibly the real life samples were affected by other air pollutant depositions and not only by the six selected heavy metals.

This discussion in relation to the soluble fraction ratios in deposition in Izmir indicated that the studied metals are ready to impose ecotoxic impacts in the water-soil environments and on biota. This impact is enhanced by highly soluble fractions in the dry deposition, too. Sudden increases in heavy metal deposition in dissolved form can be an important input into the biochemical cycles and may create more significant impacts.

Toxicity index data shows that the studied metals account for only part of the overall toxicity of the samples. This is because of the interactions between heavy metals in the form of antagonism which arose from co-existence of the metals and other toxic ingredients in aqueous samples. LUMIStox® test data provided antagonistic interactions for each heavy metal in this study.

5.2 Suggestions

Solubilities of heavy metals in wet and dry deposition samples are quite different. This may be due to the molecular differences in these two forms of deposition. In the future if the complete anion-cation analyses of the deposition samples are carried out, the variations in solubilities can be explained.

Another future work could be related to the size distribution of atmospheric particles and their chemical break-down.

In real water surfaces as receiving media for atmospheric deposition the solubilities may differ from what was found in this thesis work. The reason is that under the controlled conditions in WSS sampling (Distilled water was used) and the rainwater which is rather like distilled water, the heavy metals are dissolved at different percentages than the saline waters in the environment. Therefore real environmental water as receiving media should be used to compare the solubilities i.e. the ecotoxicities as found in this study.

Toxicities from six heavy metals is far from explaining the overall toxicities measured in this study. Then more toxic components transferring from air to water must be tested for ecotoxicity.

REFERENCES

Akkoyunlu, B.O., & Tayanc, M. (2003). Analyses of wet and bulk deposition in four different regions of Istanbul, Turkey. *Atmospheric Environment, 37*, 3571-3579.

Alagha, O., & Tuncel, G. (2003). Evaluation of air quality over the Black Sea: Major ionic composition of rainwater. *Water, Air and Soil Pollution, 3* (5-6), 89-98.

Al-Momani, I.F., Ataman, O.Y., Anwari, M.A., Tuncel, S., Köse, C., & Tuncel, G. (1995). Chemical composition of precipitation near an industrial area at Izmir, Turkey. *Atmospheric Environment, 29*, 1131-1143.

Al-Momani, I. F., Aygun, S., & Tuncel, G. (1998). Wet deposition of major ions and trace elements in the eastern Mediterranean basin. *Journal of Geophysical Research-Atmospheres, 103*, 8287-8300.

Alp, K., Çitil, E., Eldem, N., & Bayhan, H. (1999). Yoğun taşıt trafiğine sahip bölgelerde hava kalitesinin izlenmesi: İstanbul Zincirlikuyu Caddesi örneği. Hava Kirlenmesi ve Kontrolü Ulusal Sempozyumu, 23-30. 27-29 Eylül 1999. İzmir.

Agency for Toxic Substances and Disease Registry (ATSDR), (1999). Toxicological Profile for Cadmium. Retrieved February 16, 2005, from http://www.atsdr.cdc.gov/tfacts5.pdf.

Agency for Toxic Substances and Disease Registry (ATSDR), (2001). Toxicological Profile for Chromium. Retrieved February 19, 2005, from http://www.atsdr.cdc.gov/tfacts7.pdf.

Agency for Toxic Substances and Disease Registry (ATSDR), (2004). Toxicological Profile for Copper. Retrieved February 16, 2005, from http://www.atsdr.cdc.gov/tfacts132.pdf.

Agency for Toxic Substances and Disease Registry (ATSDR), (2005a). Toxicological Profile for Lead. Retrieved February 16, 2005, from http://www.atsdr.cdc.gov/tfacts13.pdf.

Agency for Toxic Substances and Disease Registry (ATSDR), (2005b). Toxicological Profile for Nickel. Retrieved February 16, 2005, from http://www.atsdr.cdc.gov/tfacts15.pdf.

Agency for Toxic Substances and Disease Registry (ATSDR), (2005c). Toxicological Profile for Zinc. Retrieved February 16, 2005, from http://www.atsdr.cdc.gov/tfacts60.pdf.

Azimi, S., Ludwig, A., Thevenot, D.R., & Colin, J.L. (2003). Trace metal determination in total atmospheric deposition in rural and urban areas. *The Science of the Total Environment, 308*, 247-256.

Azimi, S., Rocher, V., Muller, M., Moilleron, R., & Thevenot, D.R. (2005). Sources, distribution and variability of hydrocarbons and metals in atmospheric deposition in an urban area (Paris, France). *Science of the Total Environment, 337*, 223-239.

Bahadir, M., & Wundram, M. (1998). Ecotoxicological test systems for the evaluation of toxic compounds in effluents. 1st International Workshop on Environmental Quality and Environmental Engineering in the Middle East Region. S.U.Turkey.

Başak, B., & Alagha, O. (2004). The chemical composition of rainwater over Büyükçekmece Lake, Istanbul. *Atmospheric Research, 71*, 275-288.

Bayram, A., Yılmaz, Z., Odabasi, M., & Muezzinoglu, A. (1997). Levels of some heavy metals suspended particulate matter in and around Aliaga industrial region, Izmir, Turkey. *Environmental Research Forum, 7-8*, 280-285.

Bozlaker, A. (2002). Trace metals in airborne particles and their dry deposition in Izmir. MS Thesis, Graduate School of Natural and Applied Sciences, Dokuz Eylül University, Izmir, Turkey.

Brewer, R., & Belzer, W. (2001). Assessment of metal concentrations in atmospheric particles from Burnaby Lake, British Columbia, Canada. *Atmospheric Environment, 35* (30), 523-5233.

Cakan, A. (1999). The direct measurements of the dry deposition of organochlorine pesticides and polychlorinated naphthalenes. Ph.D. Thesis, Illinois Institute of Technology, Chicago, IL.

Calow, P. (Ed.). (1998). *Handbook of ecotoxicology.* USA: Blackwell Science Ltd.

Canada's National Occupational Health & Safety Resource (CCOHS), (2004). What is meant by the term "synergism"?. Retrieved March 3, 2005, from http://www.ccohs.ca/oshaanswers/chemicals/synergism.html

Chen, C.Y., Huang, J.B., Wang, Y.J., Huang, J.C., & Huang, J.W. (1996). An evaluation of microbial toxicity of effluents from petrochemical plants. *2nd Specialized Conference on Pretreatment of Industrial Wastewaters*. 65-72. Athens.

Choi, K., & Meier, P.G. (2001). Toxicity evaluation of metal plating wastewater employing the Microtox® assay: A comparison with Cladocerans and fish. *Environmental Toxicology, 16* (2), 136-141.

Codina, J.C., Munoz, M.A., Cazorla, F.M., Perez-Garcia, A., Morinigo, M.A., & Vicente, A.D. (1998). The inhibition of methanogenic activity from anaerobic domestic sludges as a simple toxicity bioassay. *Water Research, 32* (4), 1338-1342.

Conko, K.M., & Rice, K.C. (2000). Atmospheric wet deposition of trace elements to a suburban catchments in Northern Virginia: *AGU Spring Meeting*, May 2000, Washington, D.C.

Conko, K.M., Rice, K.C., & Kennedy, M.M.M.M. (2004). Atmospheric wet deposition of trace elements to a suburban environment, Reston, Virginia, USA. *Atmospheric Environment, 38* (24), 4025-4033.

Conneli, D.W., & Miller, G.J. (Eds.). (1984). *Chemistry and ecotoxicology of pollution.* School of Australian Environmental Studies. Griffith University.

Dalzell, D.J.B., Alte, S., Aspichueta, E., Sota, A., Etxebarria, J., Gutierrez, M., et al. (2002). A comparison of five rapid direct toxicity assessment methods to determine toxicity of pollutants to activated sludge. *Chemosphere, 47*, 535-545.

Deboudt, K., Flament, P., & Bertho, M.L. (2004). Cd, Cu, Pb and Zn concentrations in atmospheric wet deposition at a coastal station in Western Europe. *Water, Air and Soil Pollution, 151*, 335-359.

Dincer, F., Muezzinoğlu, A., & Elbir, T. (2003). SO_2 levels at forested mountains around Izmir, Turkey and their possible sources. *Water, Air and Soil Pollution, 147*, 331-341.

Dr. Lange (1994) *Application Report: Measurement of biological toxicity with the luminescent bacteria test, BIO NR. 101.* Düsseldorf, Germany.

Ellermann, T., Hertel, O., Skjøth, C.A., Kemp, K., & Monies, C. (2003). *Atmospheric deposition 2002.* Final Report.

EMEP Note (2003). *Convention on long-range transboundary air pollution, co-operative programme for monitoring and evaluation of the long-range* *transmission of air pollutants in Europe. Transboundary pollution by HM and POPs.* MSC-E Note 6/2003.

EMEP Report (2003). *Convention on long-range transboundary air pollution, co-operative programme for monitoring and evaluation of the long-range transmission of air pollutants in Europe. Heavy metals: Transboundary pollution of the environment.* Status Report by Ilyin, I., Travnikov, O., Aas, W., & Uggerud, H.Th.

Espinosa, A.J.F., Rodríguez, M.T., Rosa, F.J.B., & Sánchez, J.C.J. (2002). A chemical speciation of trace metals for fine urban particles. *Atmospheric Environment, 36* (5), 773-780.

European Commission Report (2002). Heavy metals in waste. Final report. Retrieved February 17, 2006, from http://www.environmental-expert.com/articles/article1208/heavymetalsreport.pdf

Fang, G.C., Wu, Y.S., Huang, S.H., & Rau, J.Y. (2004). Dry deposition (downward, upward) concentration study of particulates and heavy metals during daytime, nighttime period at the traffic sampling site of Sha-Lu, Taiwan. *Chemosphere, 56,* 509-518.

Foltescu, V.L., Lindgren, E.S., Isakson, J., Öblad, M., Tiede, R., Sommar, J., et al. (1996). Airborne concentrations and deposition fluxes of major and trace species at marine stations in Southern Scandinavia. *Atmospheric Environment, 30* (22), 3857-3872.

Gao, Y., Nelson, E.D., Field, M.P., Ding, Q., Li, H., Sherrella, R.M., et al. (2002). Characterization of atmospheric trace elements on $PM_{2.5}$ particulate matter over the New York–New Jersey harbor estuary. *Atmospheric Environment, 36*, 1077-1086.

Golomb, D., Ryan, D., Eby, N., Underhill, J., & Zemba, S. (1997). Atmospheric deposition of toxics onto Massachusetts Bay-I.Metals. *Atmospheric Environment, 31* (9), 1349-1359.

Grantz, D.A., Garner, J.H.B., & Johnson, D.W. (2003). Ecological effects of particulate matter. *Environment International, 29,* 213-239.

Grömping, A.H.J., Ostapczuk, P., & Emons, H. (1997). Wet deposition in Germany: Long-term trends and the contribution of heavy metals. *Chemosphere, 34* (9/10), 2227-2236.

Gülsoy, G., Tayanç, M., & Ertürk, F. (1999). Chemical analyses of the major ions in the precipitation of Istanbul, Turkey. *Environmental Pollution, 105* (2), 273-280.

Halstead, M.J.R., Cunninghame, R.G., & Hunter, K.A. (2000). Wet deposition of trace metals to a remote site in Fiordland, New Zealand. *Atmospheric Environment, 34*, 665-676.

Heal, M.R., Hibbs, L.R., Agius, R.M., & Beverland, I.J. (2005). Total and water-soluble trace metal content of urban background PM_{10}, $PM_{2.5}$ and black smoke in Edinburgh, UK. *Atmospheric Environment, 39,* 1417-1430.

Herut, B., Nimmo, M., Medway, A., Chester, R., & Krom, M.D. (2001). Dry atmospheric inputs of trace metals at the Mediterranean coast of Israel (SE Mediterranean): Sources and fluxes. *Atmospheric Environment, 35* (4), 803-813.

Holsen, T.M., Noll, K.E., Fang, G.C., Lee, W.J., Lin, J.M., & Keeler, G.J. (1993). Dry deposition and particle size distributions measured during the Lake Michigan Urban Air Toxics Study. *Environmental Science and Technology, 27*, 1327-1333.

Horváth, Zs., Lástity, A., Varga, I., Mészáros, E., & Molnár, A. (1994). Determination of trace metals and speciation of chromium ions in atmospheric precipitation by ICP-AES and GFAAS. *Talanta, 41* (7), 1165-1168.

Hou, H., Takamatsu, T., Koshikawa, M.K., & Hosomi, M. (2005). Trace metals in bulk precipitation and throughfall in a suburban area of Japan. *Atmospheric Environment, 39* (20), 3583-3595.

Hu, G.P., & Balasubramanian, R. (2003). Wet deposition of trace metals in Singapore. *Water, Air and Soil Pollution, 144*, 285-300.

Ince, N.H., Dirilgen, N., Apikyan, I.G., Tezcanlı, G., & Üstün, B. (1999). Assessment of toxic interactions of heavy metals in binary mixtures: A statistical approach. *Archives of Environmental Contamination and Toxicology, 36*, 365-372.

Injuk, J., Grieken, R.V., & De Leeuw, G. (1998). Deposition of atmospheric trace elements into the North Sea: Coastal, ship, platform measurements and model predictions. *Atmospheric Environment, 32* (17), 3011-3025.

Isenberg, D.L., & Bulich, A. (Eds.). (1994). *Environmental monitoring: Use of Luminescent Bacteria.* Richardson M.

Isidori, M., Ferrara, M., Lavorgna, M., Nardelli, A., & Parrella, A. (2003). In situ monitoring of urban air in Southern Italy with the tradescantia micronucleus bioassay and semipermeable membrane devices (SPMDs). *Chemosphere, 52,* 121-126.

Jaradat., Q.M., Momani, K.A., Jiries, A.G., El-Alali, A., Batarseh, M.I., Sabri, T.G., et al. (1999). Chemical composition of urban wet deposition in Amman, Jordan. *Water, Air and Soil Pollution, 112*, 55-65.

Jennings, V.L.K., Brandes, H.R., & Bird, D.J. (2001). Assessing chemical toxicity with the bioluminescent photobacterium (*Vibrio fischeri*): A comparison of three commercial systems. *Water Research, 35* (14), 3448-3456.

Kaiser, K., & Palabrica, V. (1991). Photobacterium phosphoreum toxicity data index. *Water Pollution Research Journal of Canada, 26*, 361-431.

Kanellopoulou, E.A. (2001). Determination of heavy metals in wet deposition of Athens. *Global Nest: the International Journal*, *3* (1), 45-50.

Kaya, G., & Tuncel, G. (1997). Trace element and major ion composition of wet and dry deposition in Ankara, Turkey. *Atmospheric Environment, 31* (23), 3985-3998.

Kim, G. (1998). Atmospheric inputs and upper ocean biogeochemistry of trace elements and radionuclide tracers in the Atlantic. Ph.D. Thesis, University of Delaware.

Kim, G., Scudlark, J.R., & Church, T.M. (2000). Atmospheric wet deposition of trace elements to Chesapeake and Delaware Bays. *Atmospheric Environment, 34* (20), 3437-3444.

Klaasen, C.D. (Ed.). (1996). *Casarett and Doull's toxicology: The basic science of poisons* (5th ed.). New York: Mc-Graw Hill.

Landis, W.G., & Yu, M.H. (Eds.). (1999). *Introduction to Environmental Toxicology Impacts on Chemicals Upon Ecological Systems* (2nd ed.). Florida, USA: CRC Press, Lewis Publishers.

Lin, J-M., Fang, G-C., Holsen, T.M., & Noll, K.E. (1993). A comparison of dry deposition modeled from size distribution data and measured with a smooth surface for total particle mass, lead and calcium in Chicago. *Atmospheric Environment, 27A* (7), 1131-1138.

Liu, C.L., Zhang, J., Chen, H.T., & Ren, H.B. (2003). Heavy metals in wet deposition (precipitation) from the yellowsea and the East China sea regions. *Geophysical Research Abstracts, 5*.

Luo, W. (2001). Wet deposition fluxes of soluble chemical species and the elements in insoluble materials. *Atmospheric Environment, 35*, Short communication, 2963-2967.

Maneux, E., Grousset, F.E., Buat-Ménard, P., Lavaux, G., Rimmelin, P., & Lapaquellerie, Y. (1999). Temporal patterns of the wet deposition of Zn, Cu, Ni, Cd and Pb: The Arcachon Lagoon (France). *Water, Air and Soil Pollution, 114* (1-2), 95-120.

Microbics Corporation (1995). *Microtox acute toxicity basic test procedures.* Carlsbad, CA: Microbics Corporation.

Migon, C., Journel, B., & Nicolas, E. (1997). Measurement of trace metal wet, dry and total atmospheric fluxes over the Ligurian Sea. *Atmospheric Environment, 31* (6), 889-896.

Morselli, L., Cecchini, M., Grandi, E., Iannuccilli, A., Barilli, L., & Olivieri P. (1999). Heavy metals in atmospheric surrogate dry deposition. *Chemosphere, 38* (4), 899-907.

Morselli, L., Olivieri, P., Brusori, B., & Passarini, F. (2003). Soluble and insoluble fractions of heavy metals in wet and dry atmospheric depositions in Bologna, Italy. *Environmental Pollution, 124*, 457-469.

Morselli, L., Brusori, B., Passarini, F., Bernardi, E., Francaviglia, R., Gataleta, L., et al (2004). Heavy metals monitoring at a Mediterranean natural ecosystem of Central Italy. Trends in different environmental matrixes. *Environmental International, 30*, 173-181.

Mowat, F.S. (2000). Toxicity assessment of complex contaminant mixtures in Louisiana sediments. Ph.D. Thesis. The Department of Biomedical Engineering of the Graduate School of Tulane University. USA.

Mowat, F.S., & Bundy, K.J. (2002a). Experimental and mathematical/computational assessment of the acute toxicity of chemical mixtures from the Microtox® assay. *Advances in Environmental Research, 6* (4), 547-558.

Mowat, F.S., & Bundy, K.J. (2002b). A mathematical algorithm to identify and prioritize pollutants in field sediments. *Chemosphere, 49* (5), 499-513.

Muezzinoglu, A., & C.Cizmecioglu, S. (in press). Deposition of heavy metals in a Mediterranean climate area. *Atmospheric Research.*

Nguyen, V.D., Merks, A.G.A., &Valenta, P. (1990). Atmospheric deposition of acid, heavy metals, dissolved organic carbon and nutrients in the Dutch Delta area in 1980-1986. *The Science of the Total Environment, 99* (1-2), 77-91.

Nouri, J., Alloway, B.J., & Peterson, P.J. (2001). The vertical movement of cadmium, nickel and lead through undisturbed cores having a surface amended of sewage sludge. *OnLine Journal of Biological Sciences, 1* (10), 986-988.

Odabasi, M., Sofuoglu, A., Vardar, N., Tasdemir, Y., & Holsen, T.M. (1999). Measurement of dry deposition and air-water exchange of polycyclic hydrocarbons with the water surface sampler. *Environmental Sciences, 33*, 426-434.

Odabasi, M., Muezzinoglu, A., & Bozlaker, A. (2002). Ambient concentrations and dry deposition fluxes of trace elements in Izmir, Turkey. *Atmospheric Environment, 36*, 5841-5851.

Ownby, D.R. (2002). Predicting metal interactions with a novel quantitative ion character-activity relationship (QICAR) approach. Ph.D. Thesis, The Faculty of the School of Marine Science The College of William and Mary in Virginia.

Örnektekin, Ş., Mıstıkoğlu, S., & Özyılmaz, G. (1999). İskenderun Körfezi'nde kurulu endüstrilerin hava kirlenmesine etkilerinin partiküler madde içeriği açısından incelenmesi. Hava Kirlenmesi ve Kontrolü Ulusal Sempozyumu, 222-231. 27-29 Eylül 1999. İzmir.

Örnektekin, S., & Çakmaklı, S. (2003). Chemical composition and acidity of rain at the Gulf of Iskenderun, North-East Mediterranean. *Water, Air and Soil Pollution, 3* (5-6), 157-172.

Özsoy, T. & Örnektekin, S. (2005). Elemental composition of rainwater in Mersin, an urban site in the North Eastern Mediterranean. Proceedings of the Third International Symposium on Air Quality Management at Urban, Regional and Global Scales & 14th IUAPPA Regional Conference, 18-27. 26-30 September 2005. Mövenpick Hotel-Istanbul, Turkey.

Pandey, A.K., Pandey, S.D., Misra, V., & Srimal, A.K. (2003). Removal of chromium and reduction of toxicity to Microtox system from tannery effluent by the use of calcium alginate beads containing humic acid. *Chemosphere, 51*, 329-333.

Paode, R.D., Sofuoglu, S.C., Sivadechathep, J., Noll, K.E., Holsen, T.M., & Keeler, G.J. (1998). Dry deposition fluxes and mass size distributions of Pb, Cu, and Zn measured in Southern Lake Michigan during AEOLOS. *Environmental Sciences of Technology, 32*, 1629-1635.

Pike, S.M., & Moran, S.B. (2001). Trace elements in aerosol and precipitation at New Castle, NH, USA. *Atmospheric Environment, 35,* 3361-3366.

Pirrone, N., Keeler, G.J., & Holsen, T.M. (1995). Dry deposition of trace elements to Lake Michigan: A hybrid-receptor deposition modeling approach. *Environmental Science & Technology, 29* (8), 2112-2122.

Prego, R., & Garcia, A.C. (2003). Twentieth century overview of heavy metals in the Galician Rias (NW Iberian Peninsula). *Environmental Pollution, 121*, 425-452.

Querol, X., Alastuey, A., Rodriguez, S., Plana, F., Ruiz, C.R., Cots, N., et al. (2001). PM_{10} and $PM_{2.5}$ source apportionment in the Barcelona Metropolitan area, Catalonia, Spain. *Atmospheric Environment, 35,* 6407-6419.

Querol, X., Alastuey, A., Rosa, J., Campa, A.S., Plana, F., & Ruiza, C.R. (2002). Source apportionment analysis of atmospheric particulates in an industrialised urban site in southwestern Spain. *Atmospheric Environment, 36*, 3113-3125.

Quiterio, S.L., Sousa, C.L., Arbilla, G., & Escaleira, V. (2005). Evaluation of levels, sources and distribution of airborne trace metals in seven districts of the Baixada Fluminense, Rio de Janeiro, Brazil. *Atmospheric Environment, 39* (19), 3503-3512.

Ragosta, M., Caggiano, R., D'Emilio, M., & Macchiato, M. (2002). Source origin and parameters influencing levels of heavy metals in TSP, in an industrial background area of Southern Italy. *Atmospheric Environment, 36,* 3071-3087.

Ren, S., & Frymier, P.D. (2003). Kinetics of the toxicity of metals to luminescent bacteria. *Advances in Environmental Research, 7*, 537-547.

Ren, S., Mee, R.W., & Frymier, P.D. (2004). Using factorial experiments to study the toxicity of metal mixtures. *Ecotoxicology and Environmental Safety, 59* (1), 38-43.

Rojas, C.M., Grieken, R.E.V., & Laane, R.W. (1993). Comparison of three dry deposition models applied to field measurements in the Southern Bight of the North Se*a. Atmospheric Environment, 27A*, 363-370.

Sakata, M., & Marumoto, K. (2004). Dry deposition fluxes and deposition velocities of trace metals in the Tokyo Metropolitan Area measured with a water surface sampler. *Environmental Science and Technology, 38*, 2190-2197.

Sakata, M., Marumoto, K., Narukawa, M., & Asakura, K. (2006). Regional variations in wet and dry deposition fluxes of trace elements in Japan. *Atmospheric Environment, 40*, 521-531.

Samura, A., Al-Agha, O., & Tuncel, S.G. (2003). Study of trace and heavy metals in rural and urban aerosols of Uludağ and Bursa (Turkey). *Water, Air and Soil Pollution, 3* (5-6), 111-129.

Scudlark, J.R., Conko, K.M., & Church, T.M. (1994). Atmospheric wet deposition of trace elements to Chesapeake Bay: CBAD study year 1 results. *Atmospheric Environment, 28* (8), 1487-1498.

Shahin, U.M., Zhu, X., & Holsen, T.M. (1999). Dry deposition of reduced and reactive nitrogen: A surrogate surface approach. *Environmental Science and Technology, 33*, 2113-2117.

Shahin, U.M., Holsen, T.M., & Odabasi, M. (2002). Dry deposition measured with a water surface sampler: A comparison to modeled results. *Atmospheric Environment, 36*, 3267-3276.

Shannigrahi, A.S., Fukushima, T., & Ozaki, N. (2005). Comparison of different methods for measuring dry deposition luxes of particulate matter and polycyclic aromatic hydrocarbons (PAHs) in the ambient air. *Atmospheric Environment, 39*, 653-662.

Sillanpaa, M., & Oikari, A. (1996). Assessing the impact of complexation by EDTA and DTPA on heavy metal toxicity using Microtox bioassay. *Chemosphere, 32* (8), 1485-1497.

Sofuoglu, S.C., & Holsen, T.M. (1997). Dry deposition fluxes and mass size distributions of trace elements measured near Southern Lake Michigan. *Environmental Research Forum, 7-8*, 691-696.

Sorvary, J., & Sillanpaa, M. (1996). Influence of metal complex formation on heavy metal and free EDTA and DTPA acute toxicity determined by *Daphnia Magna. Chemosphere, 33* (6), 1119-1127.

Swedish Environmental Protection Agency [Swedish EPA], (2001). *Heavy Metals.* Retrieved February 2, 2006, from http://www.internat.naturvardsverket.se/index.php3?main=/documents/pollutants/metall/tungmete.html.

Sweet, C.W., Weiss, A., & Vermette, S.J. (1998). Atmospheric deposition of trace metals at three sites near the Great Lakes. *Water, Air and Soil Pollution, 103* (1-4), 423-439.

Takeda, K., Marumoto, K., Minamikawa, T., Sakugawa, H., & Fujiwara, K. (2000). Three-year determination of trace metals and the lead isotope ratio in rain and snow depositions collected in Higashi-Hiroshima, Japan. *Atmospheric Environment, 34*, 4525-4535.

Tanner, P.A., & Wong, A.Y.S. (2000). Soluble trace metals and major ionic species in the bulk deposition and atmosphere of Hong Kong. *Water, Air and Soil Pollution, 122,* 261-279.

Tasdemir, Y. (1997). Modification and evaluation of a water surface sampler to investigate the dry deposition and air-water exchange of polychlorinated biphenyls (PCBs). Ph.D. Thesis. Illinois Institute of Technology, Chicago, IL.

Tasdemir, Y., Kural, C., Cindoruk, S.S., & Vardar, N. (*in press*). Assessment of trace element concentrations and their estimated dry deposition fluxes in an urban atmosphere. *Atmospheric Research.*

Tasić, M., Rajić, S., & Novaković, V. (2001). Atmospheric deposition of heavy metals during NATO campaign in Belgrade. Retrieved April 28, 2005, from http.//ies.jrc.cec.eu.int/Units/cc/events/torino2001/torinocd/index.htm.

Taşdemir, Y., & Kural, C. (2005). Atmospheric dry deposition fluxes of trace elements measured in Bursa, Turkey. *Environmental Pollution, 138*, 462-472.

Tatara, C.P., Newman, M.C., McCloskey, J.T., & Williams, P.L. (1998). Use of ion characteristics to predict relative toxicity of mono-, di- and trivalent metal ions: *Caenorhabditis elegans* LC_{50}. *Aquatic Toxicology, 42*, 255-269.

The Risk Assessment Information System [RAIS], (2004a). Toxicity profiles: Toxicity summary for lead. Retrieved May 8, 2005, from http://risk.lsd.ornl.gov/tox/profiles/lead.shtml.

The Risk Assessment Information System [RAIS], (2004b). Toxicity profiles: Toxicity summary for nickel and nickel compounds. Retrieved May 8, 2005, from http://risk.lsd.ornl.gov/tox/profiles/nickel_and_nickel_compounds_f_V1.shtml.

Tuncer, B., Bayar, B., Yesilyurt, C., & Tuncel, G. (2001). Ionic composition of precipitation at the Central Anatolia (Turkey). *Atmospheric Environment, 35*, 5958-6002.

Türkan, İ., Henden, E., Çelik, Ü., & Kıvılcım, S. (1995). Comparison of moss and bark samples as biomonitors of heavy metals in a highly industrialised area in Izmir, Turkey. *Science of the Total Environment, 166* (1-3), 61-67.

U.S.Environmental Protection Agency (1998). *Toxicological review of hexavalent chromium.* In support of summary information on the Integrated Risk Information System (IRIS). Washington, DC. Retrieved October 2, 2002, from http://www.epa.gov/iris/toxreviews/0144-tr.pdf.

U.S.Environmental Protection Agency (2004). *Technology Transfer Network Air Toxics Website: About air toxics.* Retrieved February 25, 2005, from http://www.epa.gov/ttn/atw/allabout.html.

Villaescusa, I., Martinez, M., Pilar, M., Murat, J.C., & Hosta, C. (1996). Toxicity of cadmium species on luminescent bacteria. *Fresenius Journal of Analytical Chemistry, 354*, 566-570.

Vinogradova, A.A. (2000). Anthropogenic pollutants in the Russian Arctic atmosphere: sources and sinks in spring and summer. *Atmospheric Environment, 34* (29-30), 5151-5160.

Voutsa, D. & Samara, C. (2002). Labile and bioaccessible fractions of heavy metals in the airborne particulate matter from urban and industrial areas. *Atmospheric Environment, 36*, 3583-3590.

Wang, W.H., Wong, M.H., Leharne, S., & Fisher, B. (1998). Fractionation and biotoxicity of heavy metals in urban dusts collected from Hong Kong and London. *Environmental Geochemistry and Health, 20*, 185-198.

Watershedss (2003). *Heavy metals.* Retrieved February 2, 2005, from http://www.water.ncsu.edu/watershedss/info/hmetals.html.

Wong, C.S.C., Li, X.D., Zhang, G., Qi, S.H., & Peng, X.Z. (2003). Atmospheric deposition of heavy metals in the Pearl River Delta, China. *Atmospheric Environment, AE International-Asia, 37*, 767-776.

World Health Organization [WHO], (2000). Cadmium air quality guidelines. Chapter 6.3. Second Edition. Copenhagen, Denmark. Retrieved February 1, 2005, from http://www.euro.who.int/document/aiq/6_3cadmium.pdf.

Wu, Z.Y., Han, M., Lin, Z.C., & Ondov, J.M. (1994). Chesapeake Bay atmospheric deposition study, year 1: Sources and dry deposition of selected elements in aerosol particles. *Atmospheric Environment, 28* (8), 1471-1486.

Wu, Y.S., Fang, G.C., Chen, J.C., Lin, C.P., Huang, S-H., Rau, J-Y., et al. (2006). Ambient air particulate dry deposition, concentrations and metallic elements at Taichung Harbor near Taiwan Strait. *Atmospheric Research, 79* (1), 52-66.

Yi, S.M., Holsen, T.M., & Noll, K.E. (1997). Comparison of dry deposition predicted from models and measured with a water surface sampler. *Environmental Sciences and Technology, 31*, 272-278.

Yi, S.M., Shahin, U., Sivadechathep, J., Sofuoglu, S.C., & Holsen, T.M. (2001a). Overall elemental dry deposition velocities measured around Lake Michigan. *Atmospheric Environment, 28* (6), 1133-1140.

Yi, S.M., Lee, E.Y., & Holsen, T.M. (2001b). Dry deposition fluxes and size distributions of heavy metals in Seoul, Korea during yellow-sand events. *Aerosol Science and Technology, 35* (1), 569-576.

Yun, H.J., Yi, S.M., & Kim, Y.P. (2002). Dry deposition fluxes of ambient particulate heavy metals in a small city, Korea. *Atmospheric Environment, 36*, 5449-5458.