論文の内容の要旨

Abstract of Dissertation

論文題目 **Characterization of toxicants in size-fractionated urban road dust using ostracod *Heterocypris incongruens* direct contact test and toxicity identification evaluation procedure**

(カイミジンコ毒性試験と TIE 手法を用いた粒径分画した都市道路塵埃の毒性要因評価)

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(本文) (Abstract)

Urban road dusts (URDs) are an important carrier of non-point pollution sources that contains a number of pollutants arising from both the anthropogenic and natural activities. Heavy metals, polycyclic aromatic hydrocarbons (PAH), polychlorinated biphenyls, perfluorinated surfactants have been reported as the main pollutant present in URDs. The chemical nature and constituents of the pollutants vary with vehicle frequency, antecedent dry/wet weather period, particle size distribution and organic matter content. Despite wide chemical characterization of URD and quantification of many chemical species, toxicity analysis by toxicity identification evaluation (TIE) procedure is lacking. Furthermore, in toxicity assessment studies, the knowledge on role played by non-targeted organic compounds that is not analyzed in routine analysis is also very limited. An earlier study has indicated that URD in Tokyo is toxic to ostracod, *Heterocypris incongruens*. However, little is known about the toxicity factors in URD from a typical urban site of concern like arterial road, residential area, parking lot area and highway roads.

This research has been aimed in characterization of the possible toxicants, mainly in the form of heavy metals, organic compounds, ammonia and non-targeted toxicants. The research is expected to provide information on the conditions on which URD becomes toxic, the roles played by particle size distribution, antecedent dry/wet weather period, traffic volume in governing toxicity. Furthermore it is

expected to provide information on toxicants wash off and build up in environment. The main objective of this research was to characterize toxicity of size-fractionated URDs in and around Tokyo, Japan by ostracod *Heterocypris incongruens* direct contact test (ISO 14371:2012 (E)). The specific objectives include 1) Toxicity assessment of various size-fractions of the URDs, 2) Identification of difference in toxicity of wet road dust and the leachate, 3) Characterization of toxicants by toxicity identification evaluation procedure and 4) Development of strategy for the characterization of non-targeted organic toxicants by high resolution Fourier transform mass spectrometry analysis.

URDs were collected from road side gutters with the help of vacuum cleaner (St. 1 to 5) and highway sweeper (St. 6 to 9 and 10). St. 9 sample was collected from the urban highway drainage pit. Samples were sieved through 2 mm nylon mesh, dried, size fractionated by sieving (250 μm, and 63 μm) and were stored in refrigerator until analysis. Quality assurance was done by analysis of the certified reference material (NIST 1643e, BCRR® 723, PAH standard mix). Every batch of toxicity test was validated by test with control sediment (OECD soil for Phytotoxkit tests, MicroBioTests Inc, Belgium)

The first step in toxicity assessment is the basic characterization of the urban road dust in order to elaborate the cause of toxicity. The concentration (mg/kg dry wt.) range of heavy metals in the total fraction (< 2000 μm) were Cr (51–384), Ni (27–123), Cu (74–418), Zn (264–2277), Cd (0.33–2.91) and Pb (11–178). This range was similar to that of earlier studies in Tokyo and Hawaii and smaller to that of Shanghai. With few exceptions, concentration of heavy metals increased with the decreasing particle size. Sum of 12 priority PAHs was in the range 0.25 – 5.26 mg/kg dry wt similar to that of earlier study in Tokyo.

Toxicity test was performed after holding the mixture of URD and moderately hard water for 1 hour (representing rapid leaching of the toxicants and initial condition of the rainfall) and 24 hours (representing slow release of the toxicants). URD showed greater than 20% lethal mortality for 1 hour treatment. The LC20 and LC50 of URDs varied from 1.6 to 49% and 3.8 to 67% respectively. Considering 20% mortality as the lethal limit of ostracod, most (5/6) of the URDs could became non-toxic (mortality < 20%) when it gets mixed with the clean sediment at 11 times the volume of the road dust. Residential sample (St. 5), highway samples (St. 6 and 9) and highway drainage pit (St. 9) was virtually non-toxic as mortality was not significantly greater than 50%. Mortality significantly ($p < 0.05$) reduced for most of the samples after 24 hours holding time. The stations which did not showed significant reduction in mortality after 24 hours holding time had higher toxic units. Toxicity was site

specific and could not be significantly correlated with the concentrations of heavy metal and PAHs in the URDs, antecedent dry weather period, vehicles frequency, weight fraction of fine particles and organic matter content. Fine particles were not always more toxic than the other fraction of coarse and medium particles. Neither of any specific size-fraction possessed significant mortality to govern toxicity of the total fraction for all the samples. Dissolved heavy metals (Zn and Cu) and un-ionized ammonia were suspected as the primary toxicants based on LC50 values available in the literatures.

The URD was found to contain both the hydrophilic and hydrophobic toxicants when toxicity test was performed after separating the wet road dust and the water leachate. The toxic strength of the leachate was decreased by increasing the water volume and also decreased by sequential extraction with water. This suggested the dilution of hydrophilic toxicants extracted from the road dust. Wet road however showed significant mortality (>20%) even when the water volume was increased to 16 times the volume of dust suggesting strongly bound organic toxicants that cannot be leached by the water. This bound toxicants acts as the principle toxicant source in contaminating benthic riverine environment. When toxicity assessment was done after various holding time (1 hour to 10 days), toxicity of wet road dust decreased, became minimum corresponding to the critical holding time and increased again. Toxicity of leachate however increased gradually. We hypothesized that 1) Toxicity of wet road dust in shorter holding time changes by physico-chemical process leading to change in speciation of toxic components to non-toxic form, 2) There could be possible exchangeability of toxicants between wet road and the leachate, 3) In longer holding time the bio-chemical process is dominant which leds in releasing toxic components from the entrapped structure of the road dust, forms new toxic components and even non-toxic components might change its speciation to toxic form.

The phase I and II approach for TIE manipulation indicated hydrophobic organic compounds and heavy metals adsorbed onto carbonaceous (XAD-4) and cationic (SIR-300) resin as the probable toxicant for St.3 sample. Zn and Cu were confirmed as the principle toxicant as it exceeded LC50 values available in the literature. Hydrophobic organic toxicants were confirmed as toxicant by elution of the XAD recovered after the end of toxicity test. The XAD elutriate obtained by methanol, dichloromethane and acetone elution showed 100, 47 and 100% mortality respectively. When eluting solvent was sequentially changed from polar to non-polar, mortality recovered in elutriate were 33% (methanol→dichloromethane) and 7% (acetone→dichloromethane) whereas mortality recovered in changing eluting solvent from non-polar to polar (dichloromethane→methanol) was 80%. This confirmed the dominance of relatively polar hydrophobic toxicants in St. 3 sample. Toxicity could be

exhibited by the unknown multiple toxicants or due to additive toxic effect of heavy metals and organic matter thereof. Application of SIR-300 though did reduce the mortality in St. 7 sample despite the concentration of analyzed heavy metals not exceeding LC50. None of the applied resins reduced mortality in St. 8 sample indicated the presence of multiple toxicants and/or extremely toxic nature of the sample.

The high resolution Fourier transform mass spectrometry analysis of the organic compounds adsorbed on XAD indicated that toxicity of road dust could be due to the presence of some compounds in a extremely higher concentration. We also note the case that toxicity culd also be due to additive toxic effect of multiple chemicals. All the probable toxic peaks that were eluted in the dichloromethane elutriate were present in the methanol and acetone elutriate. The number of probable toxic peaks in acetone elutriate was greater than that of methanol. Finally, strategy for the identification and characterization of non-targeted organic toxicants is proposed based on high resolution Fourier transform mass spectrometry analysis. The strategy includes selection of m/z peaks, elemental composition assignment, isolation of molecular species, control selection and toxicants confirmation.

The conclusions drawn from this research could be helpful for the management of urban runoff by prioritizing wet road dust in better efficiency of treatment facilities. Though representative URD samples were taken in this study, it was limited by number of sampling location for each type of road category. In order to generalize the site specific toxic nature of the road dust, it is recommended to do intensive sampling related to traffic conditions (vehicle number and speeds), temporal and seasonal variations, antecedent dry/wet weather periods, road characterization and land use pattern. Integrated toxicity assessment studies (Toxicogenomics) is recommended with battery of species (producer and consumers – e.g. algae, daphnia and fish) to link the relative toxicity to the aquatic and human health to assist in decision making process.