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Evaluation of *Daphniamagna* for the Ecotoxicity Assessment of Alkali Leachate from Concrete

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Abstract

The cladoceran *Daphniamagna* has been used as an aquatic test species in aquatic toxicology. To evaluate the aquatic toxicity of leachate from concrete, the immobilization of *D. magna* was observed after treatment of various concentrations of leachate specimens. Reliabilities of the culture condition and the experimental protocol for acute toxicity test were successfully achieved from the standard toxicity test. The leachates were prepared from the mixture of Ordinary Portland Cement (OPC) and pozzolanic admixtures, Pulverised fuel ash (PFA), Ground granulated blast furnace slag (GGBS) and GGBS containing loess. Acute toxicity test showed 100% immobilization of *D. magna* for OPC or PFA. The leachates from OPC or PFA had high pH 10 to 12. However, GGBS and GGBS containing loess showed less toxicity according to the concentrations. Especially, immobilization was not observed at the concentrations below 12.5% of GGBS containing loess. Also the range of pH for these specimens was 8 to 9. This suggested that the use of loess as the admixture in concrete may be useful to reduce eco-toxicity of leachates from concrete. This our study provided the harmfulness of the alkali leaching from concrete in aquatic environment and the usefulness of *D. magna* to evaluate the toxicity of leachates from concrete.

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Introduction

The detection and presence of toxic materials in environment is one of the most popular interests because of their harmful effects on ecosystem including human. Especially, the presence of toxic materials in aquatic environment is more considerable to evaluate the harmfulness. The evaluation of contamination in aquatic environment has been for a long time performed only by specific chemical analyses, but many experiences and studies have shown the inadequacy of such approach. To complement this chemical analysis, the biological methods using fish or algae have been used to evaluate and monitor the water quality for many years (Sandbacka *et al.*, 2000; Versteeg *et al.*, 1997). The

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cladoceran *Daphniamagna* Straus has been used as a "standard" aquatic test species among cladoceran and most frequently used for chronic and acute tests in aquatic toxicology (ASTM, 1987; EEC, 1992; OECD, 1992). Many countries are using aquatic toxicity tests as part of their water quality monitoring program (Slabbert and Venter, 1999). In Korea also, the *D. magna* has been used for the assessment of toxicity in aquatic environment from 2011 (KEC).

The *D. magna* is a species of *Daphnia* and distributed in North America, Eurasia and some regions of Africa. This has many advantages as an experimental animal like as small, short life span and easy culture (Martins *et al.*, 2007). They can be grown in small bottle or in mass culture in water containing bacteria or their equivalent for food. Daphnia provide enough satisfactory for toxicity testing because they are food for many fish, fishes do not survive long in waters where their food supply has been destroyed.

The concrete is composed of primarily of cement, aggregate, and water and is widely used for making architectural structures, foundations, walls, bridges, dams and etc. Especially, the cement, commonly Portland cement, serves as a solidified and hardened into rock-hard strength through a chemical process. Concern about the environmental impacts of cement-based materials has been grow consistently now (Dell'Orso *et al.*, 2012; Napia *et al.*, 2012). As organic components in cement are usually burned away during formation process, they are not considered carefully with harmful materials. The primary interest of cement-based materials for the environmental impacts is the leaching of inorganic compounds when they are contact with waters (Napia *et al.*, 2012). The cement contains heavy metals and other toxic inorganic components. These components act as toxic materials in environment when they are released by water. Therefore, many studies have been focused to prevent or reduce the release of them to environment (Brunori *et al.*, 2001; Dell'Orso *et al.*, 2012; Napia *et al.*, 2012). Additionally, the alkali leaching from concrete by contacting water is most serious inorganic component, but its assessment for environment has not been studied yet. The alkali leaching is not only problem to architectural structures but also more serious to the construction of bridges, dams and other offshore structures (Van Gerven *et al.*, 2007; Mohamed and El Gamal, 2011).

The purpose of this study, therefore, was to evaluate the usage of eco-toxicity assays with *D. magna* in water contaminated with alkali leaching of concrete. To reduce toxicity, in additionally, the effect of loess as the eco-friend admixture was evaluated.

Materials and Methods

Culturing of *D. magna*

Daphniamagna was obtained from Korea Envi-

Table 1. Preparation of synthetic freshwater using reagent grade chemicals

Contents	Concentration (mg//L)
KCl	8 mg/L
$MgSO_4$	120 mg//L
$CaSO_4.2H_2O$	120 mg//L
$NaHCO_3$	192 mg//L

Table 2. Culture conditions for *D. magna*

Items	Conditions
Lighting	900Lux ~1000Lux "Cool white" Fluorescent 16h light : 8h dark
Temperature	20±1°C
Hardness	160~180 mg/L
pH	7.6~7.8
Prey	Pseudokirchneriella sp.

ronment Corporation (KEC), Korea, and maintained in laboratory by the standard culturing method suggested by KEC (Table 1) (KEC). The synthetic hard water for culture was manufactured as Table 2. *Daphnia magna* was cultured individually in 100 ml glass beaker containing hard water and fed with $2\sim2.8 \times 10^5$ cells/15 ml concentrated algal solution of *Pseudokirchneriella* sp.

Alkali leachate

The alkali leaching specimen was prepared from the mixture of Ordinary Portland Cement (OPC) and pozzolanic admixture. The specimens were made with not only cement and water but also the admixture instead of cement. The ratio of cement to water was 10:3, 10:4 and 10:5, respectively. The admixture replacement percentage were followed in the 10:4 ratio of cement to water; 30% Pulverised fuel ash (PFA), 60% Ground granulated blast furnace slag (GGBS) and 60% GGBS containing loess, respectively. Each specimen was treated to the sealed container filled with water for 30 days, and then the leachate was prepared from it.

Standard toxicity test

As a means of controlling the sensitivity of *D. magna* and the reliability of the experimental protocol, an internal control test was performed with each series of tests in standard test conditions; potassium dichromate ($K_2Cr_2O_7$) was used as a reference toxicant. The test was performed with nominal concentrations of 0.5, 1.0, 2.0, 3.0 and 4.0 mg/L. In each test item concentration four replicates were maintained and each containing 5 neonates in 50 ml of medium. The immobilized or died neonates were recorded at 24 hrs of exposure. EC_{50} values to *D. magna* were determined by probit analysis (Finney, 1971). Whenever the EC_{50} value of *D. magna* fell outside the allowed range, the data of the entire test series were considered unacceptable and the experiment was repeated.

Acute toxicity test

All experiments were carried out with brood neonates (< 24 hrs old). Twenty *D. magna* per treatment were used and not fed during tests. Temperature and photoperiod were as described in Table 2. The test solutions were diluted by hard water with concentrations of 100, 50, 25, 12.5 and 6.25%. In each concentration four replicates were performed and each containing 5 neonates in 50 ml of test solution. The measured effect was recognized as immobilization for 15s after stimulation by a bright light under microscope. After determining 24-h EC_{50} value, toxicity unit (TU) was calculated as follows, $TU = 100/EC_{50}$ (Ra *et al.*, 2008).

Results and Discussion

Standard toxicity test

To evaluate the reliability of the experimental protocol for acute toxicity test, the standard toxicity test was carried out using potassium dichromate. As the result, EC_{50} value was 1.01 mg/L at 24 hrs of exposure (Fig. 1). This value belonged to normal range (0.9~2.1

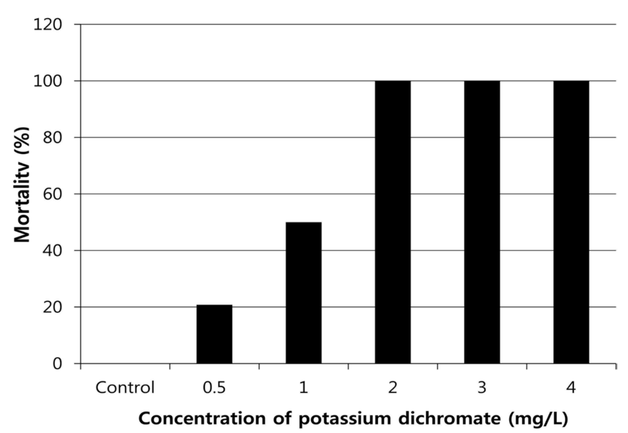

Fig. 1. Mortality test of *D.magna* using potassium-dichromate. In each test item concentration four replicates were maintained and each containing 5 neonates in 50 ml of medium. The immobilized or died neonates were recorded at 24 hrs of exposure.

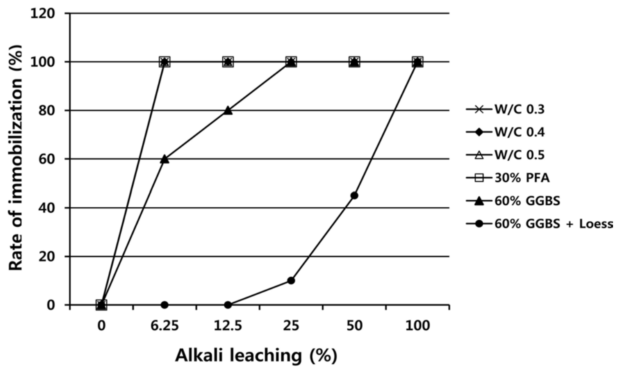

Fig. 2. Immobilization rate of *D. magna* according to the concentrations of alkali leachates from concrete. In each concentration four replicates were performed and each containing 5 neonates in 50 ml of test solution. The measured effect was recognized as immobilization for 15 s after stimulation by a bright light under microscope.

mg/L) of standard toxicity test using *D. magna* (KEC). This result indicated that the culture condition of *D. magna* and the experimental protocol are enough to satisfy the reliability of acute toxicity test.

Immobilization of *D. magna* by alkali leachate from concrete

The effect of alkali leachate from various kind of concrete to *D. magna* was determined by acute toxicity test. The result showed 100% immobilization of *D. magna* when treated with leachates from the OPC only used concrete in all concentrations (Fig. 2). This indicated that the highly toxic materials are leached from OPC. Additionally, as the pH of leachates from OPC was high from 10 to 12, we could not exclusive possibility that this high pH may be the lethal factor because the survival tolerance pH is 7 to 8.5 generally for organisms including Daphnia (Table 3). The leachate from concrete replaced with PFA 30% also showed 100% immobilization and high pH (10-12) in all concentrations. However, the replacement of OPC with GGBS 60% was effective to reduce the toxicity. Immobilization was 100% at the concentrations of above 25% of leachates from GGBS but was 60 and 80% at the concentrations of 6.25 and 12.5%, respectively. Although the leachates from GGBS showed high pH range (9-12) and this pH range was also inappropriate for the survival of *D. magna*, the survival rate was increased (Fig. 2). This result suggested that the lethal factor of *D. magna* is not only the high pH but also other toxic materials from OPC, PFA or GGBS. Because GGBS was less toxic to *D. magna*, the leachate was prepared from the concrete containing GGBS 60% and loess which is known as eco-friendly material. As the result, immobilization was observed at the concentration of

Table 3. Acute toxicity test of alkali leachates using *D. magna*.

Leachate (pH)	EC50			EC95			Toxicity unit
	conc.	lower	upper	conc.	lower	upper	
W/C 0.3 (10~12)	ND	ND	ND	ND	ND	ND	ND
W/C 0.4 (10~12)	ND	ND	ND	ND	ND	ND	ND
W/C 0.5 (10~12)	ND	ND	ND	ND	ND	ND	ND
60%GGBS (9~12)	5.654	2.337	7.507	14.849	10.811	47.513	17.67
30%PFA (10~12)	ND	ND	ND	ND	ND	ND	ND
60% GGBS + Loess (8~9)	49.058	39.065	59.152	90.200	71.510	157.844	2.04

25, 50 and 100% of leachates with 10, 45 and 100%, respectively. The pH of leachates from this concrete was also decreased to 8-9. This result indicated that the addition of loess can reduce the pH and aquatic toxicity significantly.

Acute toxicity of alkali leachates from concretes

From the preceding results, 24h-EC_{50} value and TU were calculated (Table 3). We could not determine any value of 24h-EC_{50} value and TU for the leachates from OPC only or replacement with PFA 30% because of their high toxicity. The replacement with GGBS 60%, on the other hand, showed 17.67 TU and the addition of loess, especially, reduced TU significantly to 2.04.

The amount of leaching from concrete used in this study may be much more than that from natural condition because the less water used to leach from concrete in this study. However, 100% immobilization in all concentrations of OPC or PFA 30% suggested that the effects of leachates from concrete in natural condition may be significant in aquatic environment. The strength of concrete is determined generally by the rate of cement to water. Therefore, the use of higher amount of cement in mixture is the basic essential to satisfy strength of concrete. Acute test showed significantly high toxicity to *D. magna* at the 10 to 4 ratio of cement to water which is generally used ratio in concrete (Fig. 2, Table 3). This result suggests that the development of methods to reduce the amount of cement in concrete or the alkali leaching from concrete is required for the aquatic environment. The use of PFA and GBBS as replacement of cement were not also effective to reduce the toxicity of leachate. The GBBS containing loess, however, showed remarkable effect to reducing toxicity. Loess has been used traditionally in construction of architectural structures in Korea and is considered as eco-friendly material (Kim *et al.*, 2007). As the similar material with loess, there are several reports about the effects of metakaolin for the enhancement of durability and strength of concrete (Cyr *et al.*, 2102; Gallego-Perez *et al.*, 2011; Lau *et al.*, 2003). Metakaolin is made from kaolin, and its effects for concrete are valid after activation of kaolin. Similar effects of loess were also reported in Korea (Kim *et al.*, 2007). Loess was not useful for concrete as itself, but it was effective after activation of loess by heat treatment. The use of loess as the replacement of cement in concrete, therefore, may be useful to reduce eco-toxicity of leachates from concrete after further study of loess as admixture in concrete.

In conclusion, this study provided the harmfulness of the alkali leaching from concrete in aquatic environment and the usefulness of *D. magna* to evaluate the toxicity of leachates from concrete.

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