

Evaluation of Bioavailability of Heavy Metals in Soil by *in vitro* Screening Test

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The bioavailability of cadmium, lead, and chromium leaching from soil was determined by an *in vitro* experiment utilizing artificial stomach and small intestine solutions. The dissolved concentrations of these metals in the model stomach + small intestine condition was lower than those found in the model stomach as a result of the re-adsorption to the soil by titration from pH 2.0 to 7.0.

In Japan, the Ministry of the Environment conducted their own risk assessment of hazardous materials present in soils.¹ Their approaches were based on following two pathways of hazardous materials to human body. One is ground water through a water system, the other is directly ingested soils through gastrointestinal tract.²

A study on the bioavailability of ingested mine waste was conducted by A. Davis et al.³ In this study it was found that the toxicity of ingested heavy metals depends largely on its bioavailability. This is based on toxicological studies using pure metal salts. However, the approach could be considered flawed due to the fact that natural materials in solid phases are much less soluble than metal salts. This fact causes an overestimation of the ingestion of heavy metals into organs. Bioavailability is defined as the fraction of an administered dose that reaches the central (blood) compartment from gastrointestinal tract as indicated by Critical Review.⁴ SETAC (Society of Environmental Toxicology and Chemistry) points out that potential environmental or human health risks need to be addressed using more discriminate soil metal determination techniques based on bioavailability considerations.⁵ Considering international regulation of a risk on heavy metals in soils, an evaluation based on bioavailability whether hazardous contents are dissolved and absorbed in gastrointestinal tract will be focused and investigated.

A sample was collected from 3 m depth from the surface of a waste disposal site where slag, plastic, and sludge were land-filled. The sample was air-dried and sieved to <75 μm , such a small level was chosen because smaller particles have a greater chance of adhering to children's hands and in turn being ingested.⁶

Contents of cadmium, lead, and chromium were investigated to compare with dissolved concentrations by the *in vitro* test to evaluate bioavailability. They were extracted from samples using nitric acid and hydrochloric acid by using a microwave oven, and were then filtered through a membrane filter with a diameter of 0.2 μm .

Drexler investigated the bioavailability in human through an *in vitro* test using a liquid-to-solid ratio 100:1.⁴ A liquid-to-solid ratio of 100:1 was selected for the experiment as well. The *in vitro* test to evaluate bioavailability was developed referring to previous studies.^{3,4,6,7} The fluid in the model stomach

condition was adjusted to pH 2.0 with hydrochloric acid, pepsin and organic acids such as acetate, citrate, lactate, and malate were added. A pH level of 2.0 is the optimum pH level for pepsin, which is digestion enzyme found in the stomach of human. The flasks were placed on a wrist-action shaker in a water bath at 37 ° for 2 h. After the 2 h, the reaction mixtures were titrated with sodium hydrogen carbonate to pH 7.0, pancreatin and bile extract were added to the model small intestine. The flasks were returned to the water bath. After 4 h, suspended material was removed by filtering samples through 0.2- μm membrane filters. The liquid fractions were acidified with nitric acid and heated. The concentrations of cadmium, lead, and chromium were determined by ICP-OES (Seiko Inc. SPS1700).

Figure 1 shows the contents of cadmium, lead, and chromium in the soil. Cadmium, lead, and chromium contents were 18.2, 1074, and 3797 mg/kg dry weight of soil, respectively. The dissolved concentrations of these metals in the model stomach condition were higher than those found in its stomach + small intestine counterpart as shown in Figure 2. These results indicate that heavy metals dissolved under simulated stomach condition with pH 2.0, decreased under simulated small intestine condition upon titration from pH 2.0 to 7.0. Released metals might be re-adsorbed to particles of the soil in the condition of neutral pH in the small intestine. In general, 90% of materials into the gastrointestinal tract are absorbed in the small intestine and 10% in the stomach and large intestine. Considering this point, released amounts in the model stomach + small intestine condition will be the most part likely to be absorbed.

Figure 3 shows ratio of released amounts of each metal from soil-to-contents in the soil. The released amount was calculated by multiplying the liquid-to-solid ratio of 100:1 by the dissolved concentration. Ratio of each metal decreased 37.4 to 33.5%, 65.5 to 35.0%, and 28.0 to 26.3%, respectively. The ratio suggests that their released amount in the stomach + small intestine solution was less than half of the content. Even though

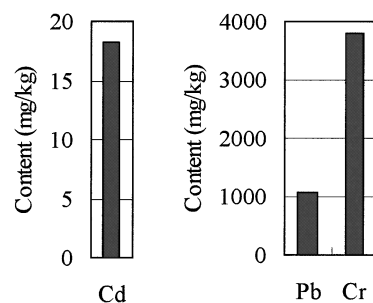


Figure 1. The contents of cadmium, lead, and chromium in soil from landfill site.

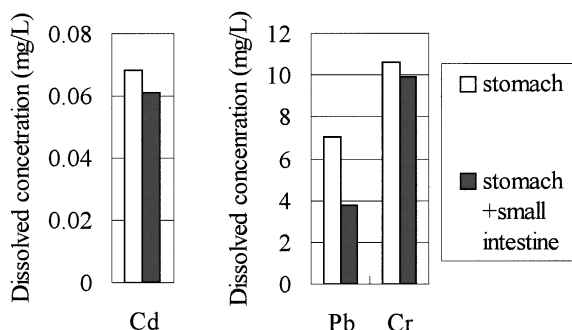


Figure 2. Dissolved concentrations of cadmium, lead, and chromium in the model stomach and stomach + small intestine condition from soil.

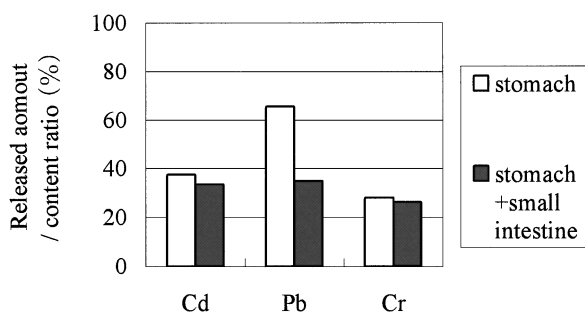


Figure 3. Ratios of released amount from soil-to-content in soil.

the soil is ingested, heavy metals dissolve in stomach with a low pH, adhere to particles in the small intestine with a neutral pH. These results confirm that the risk evaluation of exposure in organs to ingestion of heavy metals by only their content may lead

to be overestimated. Different ratios were obtained from each metal. It can be assumed that the release in organs will depend on the type of heavy metals. These results derive that the risk evaluation of exposure to organs of heavy metals based on the content of these metals present in soil dose not reflect an actual solution in human gastrointestinal tract.

Results from an in vitro screening test for bioavailability of heavy metals show that released amounts of soil in the stomach and small intestine are less than contents, a ratio of released amount-to-content are different in elements. They indicate that neutral pH in the small intestine makes released heavy metals in the stomach with a low pH adsorb to particles of the soil again. We concluded that a risk evaluation of the direct intake of soil need an evaluation based on not only content but also on bioavailability considerations.

References and Notes

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