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Assessment of the Toxicity of Stabilized Sludges Using *Hyalella curvispina* (Amphipod) Bioassays

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A wastewater treatment plant produces two "effluents", one water and the other sludge. Historically, design engineers have had a tendency to concentrate on cleaning the water while considering sludge as a secondary issue. In reality, dealing with sludge is often a more difficult challenge, both technically and operationally. Also from a cost standpoint sludge related facilities are nearly 50 % of the total cost (WEF 1987; WEF 1993).

Before the final sludge disposition there are three treatment process: thickening, stabilization and dewatering. Sludge use depends mainly of the kind of stabilization utilized. The more commonly used wastewater sludge stabilization processes are: anaerobic digestion, aerobic digestion, composting and lime stabilization. The stabilization criteria include volatile solids reductions, specific oxygen uptake rate and pathogen indicator organism reductions.

Wastewater sludges have been applied to land for soil enhancement, crop production, reclamation of disturbed lands and also as a sludge disposal method. Generally sludge which will be land applied is stabilized by anaerobic or aerobic digestion or lime treatment. The common environmental concerns for sludge land applications are nitrate migration to aquifer and surface water, heavy metal buildup in soils, metal toxicity to plant and potential harm to humans and animals, pathogens and toxic organics. To deal with these issues assessment methods are needed. Generally, sludge assessment by chemical analysis or its leachates is the more common approach. That is to evaluate some parameters which should be within of permitted limits for Toxicity Characteristic Leaching Procedure (TCLP) (Manahan 1990). The permitted TCLP limits, however, do not have a validated relationship with bioavailavility and toxicity. Because of this, final disposition of sludges requires knowledge about the kind of suitable soils, its treatment to make it impermeable or choice sites to take leached samples, in a continuous leached quality monitoring.

Prevent groundwater pollution is easier and less expensive that remediate it, chemical analysis on sludges can be difficult, requiring sophisticated and expensive methods. Thus the number and frequency of samples analyzed is lower thereby reducing precision and power.

The toxicity of sludge can be evaluated by biological methodologies which

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provide data on bioavailabitity; therefore, environmental harm can be more readily determined. In this work we show the results of direct toxicity evaluations of sludge from: tanning, agrochemical, brewery and food industries. All these use lime sludge stabilization and landfills as final disposition.

MATERIALS AND METHODS

Amphipods were collected from a population at an artificial pond at the University of Luján. They were placed in a laboratory tank (500 L) with a continuous flow of groundwater (126 mg CO_3Ca/L hardness, 800 µS/cm conductivity, 8.26 pH, 412 mg CO_3Ca/L alkalinity). Before testing, amphipods were cultured under assay conditions and age selection was done by sieving through several mesh sieves obtaining: 2300 µm / > 30 d old or adults; 600 µm / > 15 d old, 400 µm / 10-l5 d old and 200 µm / < 7 d old. Ten to fifteen d old individuals were chosen because of its relative sensitivity, 96 h LC50 0.31 (0.12-0.53) mg potassium dichromate/L and ease of handling. Age control was made using the equation:

$TL = 0.71 + t\ 0.037$

where TL is the total length in mm and t is time in d (Simionato et al. 1997). Dilution water quality was: 82 mg CO_3Ca/L hardness, 500 µS/cm conductivity, 8.3 pH, 212 mg CO_3Ca/L alkalinity (Borgmann 1996). Others conditions were 21 +l°C of temperature and 12 hours dark-light photoperiod. Sludge test conditions were:

a) Stabilized sludge and a control
b) Heat treatment at 103°C for 24 h
c) Dry homogenization and sieving at 1000µm
d) Homogenization with dilution water 1:0.5
e) 50 gr/250 mL overlying water
f) 8 replicate of ten < 14 days old *H. curvispina* each
g) Ten days static 24 h renewal tests at 21 ±l°C and 12 h photoperiod
h) Mortality recorded and test termination

Sediment from a non-polluted stream containing less than 5 % of volatile solids was used as a control.
In addition, 96 h leachate toxicity testing with *H. curvispina* was performed on all sludges. Sludges from Tannery, Brewery, Agrochemicals production and food processing industries were analyzed. All sludges assayed were subjected to lime stabilization and then were landfilled. Volatile solids were measured according APHA (1992). Sludges were collected as a composite sample from dewatering process. Each one was composite of four samples taken at regular intervals of three hours. They were stored at 4°C for non longer than two weeks.

One factor ANOVA in conjunction with Dunnet's test was used to compare responses with the control by computer program Toxstat V 3.4 (Sokal y Rohlf 1979). Regression analysis was conducted comparing between mortality and volatile solids and mortality and conductivity using Statistica.

RESULTS AND DISCUSSION

Table 1 shows physicochemical parameters of the dilution water after 24 h contact with sludges and sediment control as well as volatile solids content. Conductivity showed the greatest change of these parameters. On the other hand, leachate assays for all sludges did not show mortality at ten d. However, exposure of amphipods in whole sludges resulted in toxicity. Tannery and brewery sludges showed significant mortality at $p < 0.05$ respect to the control. Mortality was plotted against conductivity and volatile solids content as showed in figure 1 and 2.

The mortality of amphipods was correlated with conductivity and volatile solids (VS) of sludges (Figure 1 and Figure 2). The latest showed highest correlation coefficient ($p < 0.05$) which could indicate that whole sludges toxicity would be related with the VS. However, sludges that were toxics respect to the control showed high conductivity as well as high VS. On the contrary, sludge from food industries showed not significant ($p < 0.05$) mortality, even though its high conductivity values, but low VS. High conductivity alone would not explain

Table 1. Physico-chemical parameters of dilution water after 24 h in contact with sludges and sediment control.

Parameter	pH	Dissolved oxygen % saturation at 21°C	Hardness mg CO_3Ca/L	Conductivity µS/cm	Alkalinity mg CO_3Ca/L	Volatile Solids %
Control 1	8.3	55	91	500	289	6
Control 2	8.3	60	84	560	300	4
Tannery 1	7.9	50	100	3200	440	90
Tannery 2	8	55	105	3500	640	50
Agrochemical 1	8.2	55	170	700	180	35
Agrochemical 2	8.1	55	160	800	190	27
Brewery 1	8.3	60	130	1500	230	47
Brewery 2	8.1	60	130	1200	200	58
Food 1	8.2	55	140	1900	340	20
Food 2	8.7	55	128	2100	329	27

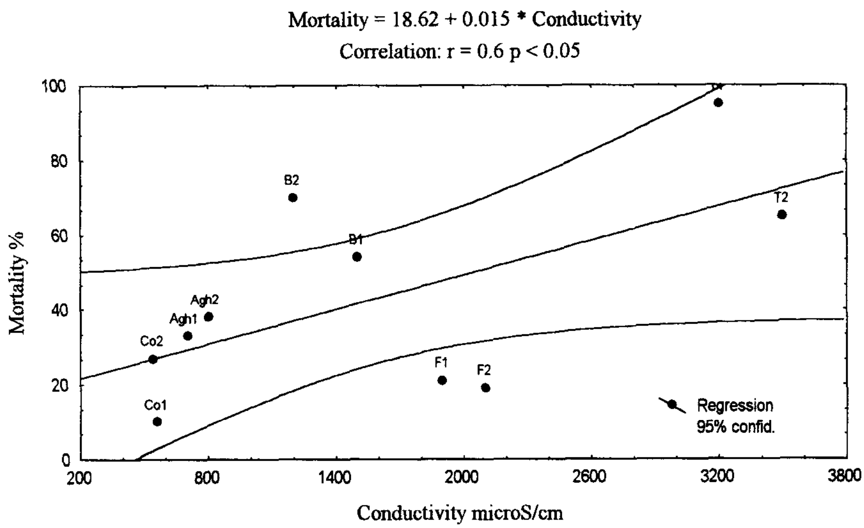

Figure 1. Regression analysis between mortality (%) and conductivity µS/cm.

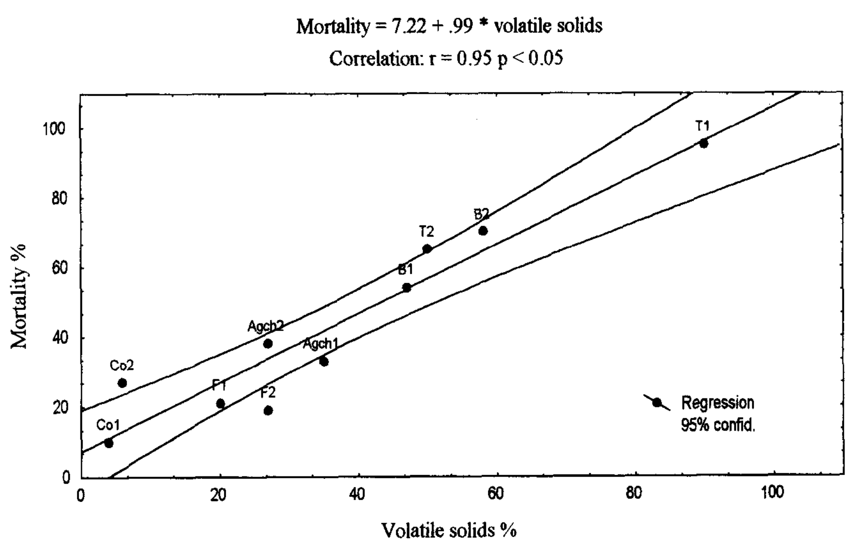

Figure 2. Regression analysis between mortality (%) and volatile solids in sludges (%).

mortality data. Likely sludge toxicity is due to a combined effect between VS and conductivity. VS would play a more important role as has been found in this study according to the correlation coefficient obtained from regression analysis.

Is has been reported that the toxicity of sediments is largely due to interstitial water (Swartz and Di Toro 1997). Organic matter plays an important part in determining chemical bioavailability. Higher organic matter contents increase the chemical sorption reducing their final concentration in the interstitial water (Power and Chapman 1992; Swartz and Di Toro 1997). A process of sludge stabilization should inactive it, chemically and biologically. So, we could expect that a stabilized sludge (or water leached) has not interstitial water toxicity. We found that in the case of leachate, at less for ten d, the later statement was true. The leaching of unstabilized sludge of these industries was toxic to *Hyalella carvispina* (unpublished data). On the contrary, leachates after lime stabilization process were not toxic. So, the lime stabilization process appears to reduce sludge toxicity.

Lime stabilization has a simple theory, essentially the pH is raised by adding lime, at a pH of 12 or more, with sufficient contact time, pathogens are destroyed and chemical and physical characteristics of the sludge are also altered by the reactions that occurs. The effects of incomplete stabilization are not readily apparent and may not be seen at the treatment plant, so proper process control techniques are very important.
Volatile solids content has long been used as a measure of the amount of organic matter in sludge. The degree of VS destruction in sludge by a sludge treatment process measures its effectiveness to stabilize its organic content (WEF 1993). Sludges, which will be land applied, are stabilized by anaerobic or aerobic digestion or lime treatment. Both anaerobic and aerobic sludge digester performances have traditionally been measured by percent reduction of VS. Anaerobic or aerobic digestion processes can remove not all VS present in the sludge. It has been suggested that only 50 to 75 % of the VS in municipal wastewater sludge are readily biodegradable. The remaining organic components in sludge consist of relatively stable carbohydrates and humus-type materials (WEF 1993). Lime stabilization of sludge usually reduces about 10 to 35 % in VS content. These could be a result of lime reaction with nitrogenous components of the sludge producing volatile compounds (WEF 1993).

We reported a relation between mortality percentage of *Hyalella curvispina* and volatile solids percentage in the assayed sludges. We think this may be a result of the introduction of chemicals during sludge feeding and/or an incomplete stabilization process with respect to volatile solids content. Also sludge toxicity could be due to high ammonia concentrations as a result of lime reaction with organic matter. The results obtained would be useful for determine the potential hazard of each sludge when it is released to the environment as well as to determine the efficiency of the stabilization sludge process.
Amphipods are frequently used as test organisms in sediment toxicity testing (Cano et al. 1996, US EPA 1994). The bioassay with *Hyalella curvispina* was shown in

this study to be useful in evaluating sludge toxicity. This will aid in assessments of environmental impact and sludge disposal.

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