SPECIATION STUDIES AND TOXICITY ASSESSMENT OF COMPLEX HEAVY METAL MIXTURES

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ABSTRACT The Microtox™ bioassay and polarographic techniques were used together to identify specific oxidation states and toxicity of metals. The bioassay is based on light reduction by bioluminescent bacteria upon exposure to toxicants. In polarography, a mercury drop substrate's potential is changed, and the substance of interest is electrochemically reduced. Reduction current is proportional to its concentration. The toxicity of solutions containing heavy metal pollutants was measured. Mercury was found to be most toxic with an acute one minute EC_{50} of 0.0162 mg/l. Cu(I) was least toxic. Speciation effects were observed; e.g., Cr(III) was less toxic than Cr(VI); Cu(II) was more toxic than Cu(I). Polarography (which is usually not used for multi-element analysis) has been extended to Pb(II) and Cd(II) solution mixtures. Various mixtures were tested to determine if toxicity was predictable from that of individual components, or whether synergistic/antagonistic reactions occur. The resultant EC_{50} for a 50-50 As(V)/Cd(II) mixture was consistent with additive behavior; Pb(II)/Cd(II) and Pb(II)/Cu(I) mixtures exhibited antagonistic and synergistic interactions, respectively. Sediments soaked with Pb(II) and Cr(III) have been studied to determine the toxicity. For competitive sorption, the EC_{50} value is twice that for Cr(III) alone, presumably because preferential Cr(III) adsorption occurs, blocking Pb(II) adsorption to kaolin.

KEYWORDS: Microtox™, heavy metal mixtures, speciation, polarography

INTRODUCTION

In recent years, concern over the presence and toxic effects of chemicals in the environment has increased dramatically. However, the determination of acute, sub-lethal, or chronic toxicity of any single compound can be very expensive and time consuming. Additionally, contaminants are often present in the environment in mixtures of unknown composition, making the task of assessing hazards even more difficult.

Thus, mixtures of hazardous pollutant substances represent vexing environmental problems. There are several important broad issues to consider in this regard, primarily involving identification of the chemical species present and assessment of the potential toxicity of the mixtures themselves. These issues are in fact highly linked in that different chemical forms may substantially vary in toxicity. For example, trivalent chromium has a low order of toxicity, while hexavalent chromium is highly toxic [1]. Therefore, speciation studies may be required to understand the potential hazards associated with a chromium mixture. In addition, the toxicity of pollutants in a mixture may differ from an additive response since synergistic or antagonistic interactions between two components in a mixture may make the mixture more toxic (or less toxic) than predicted by summing the effects of each individual toxicant. Also, prediction of the environmental hazard posed by heavy metals can be affected by complexation with organic contaminants. In this research project, the complex mixture problem is being attacked using a

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combination of toxicity assays and electrochemical methods.

Microbial tests have been widely used in toxicity screening procedures due to several factors. These include the similarity of complex biochemical functions with higher organisms, ease of handling, short exposure time, and reproducibility of results between laboratories. Bacterial enzymatic activity, growth inhibition, reproduction rate, transformations of carbon, nitrogen, or sulfur, oxygen demand, glucose uptake, and metabolic light and heat release, have been measured as parameters to assess the toxic effects of industrial wastes and single contaminants [2, 3].

Bioluminescent bacterial assays have been successfully used previously in determining the toxicity of aquatic samples, sediments, and soils. The bioassay is based on the reduction of light emitted by a nonpathogenic strain of bacteria upon exposure to a toxic sample. In this investigation, the Microtox™ system is being used to gauge the hazards of components in heavy metal mixtures.

THEORY

Microtox™

Since the first experiments in which the effect of air pollutants on luminescent bacteria was determined, the measurement of the emission of light by such organisms has been used to develop a sensitive test for the quick assessment of aquatic toxicity. The luminescent bacterial bioassay has also been successfully used in determining the toxicity of solvent extracts from sediments and soils [3, 4]. In luminescent organisms, light emission usually results from the interaction of the enzyme luciferase, reduced flavin, and a long chain aldehyde in the presence of oxygen and constitutes part of the cell's electron transport system [2, 5]. The emission of light depends on this flow of electrons, and therefore the light output reflects any change in the metabolic activity and "health" of these organisms.

The use of the light-emitting bacterium *Vibrio fischeri* for toxicity analysis was proposed in 1979, and the inhibition of light output from this microorganism is the basis of the Microtox™ method. *V. fischeri*, which exhibits decreased light production when grown in the presence of sub-lethal concentrations of toxicants, is a marine bacterium whose light emission spectrum (420 to 630 nm) has an intensity maxima at 490 nm in the visible region [2]. Intensity of light output depends on several external factors including temperature, pH, salinity, nature and concentration of the toxicants, exposure time, and age of the bacteria [2, 4].

A lyophilization procedure has been developed to standardize the bacterial culture and results have been presented on the precision, accuracy, and sensitivity of the method, and on the effect of pH, temperature, exposure time, and sample concentration on the response of the test organisms [2]. The Microtox™ system is currently used for various toxicity analysis applications including air pollution, biological toxins, bioreactivity (toxicity of medical products and biomaterials), drinking water quality, ecotoxicology, industrial and municipal effluent, hazardous waste storage, mutagenicity, petrochemical, pulp and paper, and sediment analysis.

This luminescent microorganism exhibits decreased light production when grown in the presence of sub-lethal concentrations of toxicants. Any alteration of metabolism affects the intensity of the organism's light output. Using a photometer to sense these changes in light output, the hazards

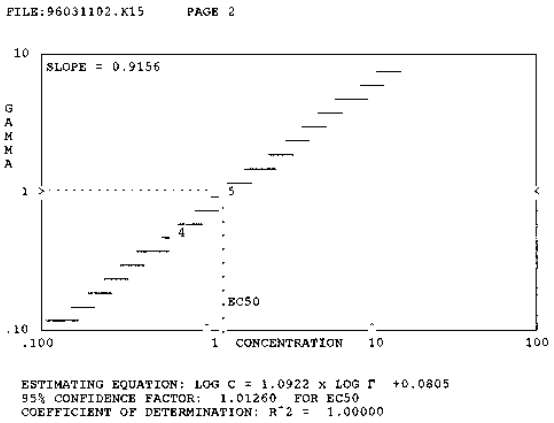

FIGURE 1. MICROTOX™ RAW DATA SAMPLE. GAMMA VALUE IS THE RATIO OF LIGHT LOST AT TIME, T, TO LIGHT REMAINING AT TIME, T, FOR A GIVEN SAMPLE CONCENTRATION.

presented by the toxicants can be obtained by establishing the EC_{50} level from data (taken with a range of toxicant concentrations), which is usually graphed. A typical raw data sample is shown in Figure 1. The EC_{50} value obtained represents effective concentration 50%, meaning that concentration of the toxicant is causing a 50% reduction in light from the baseline level with no toxicant, and is dependent on the test temperature and exposure time.

Several authors have investigated the correlation of Microtox™ to other accepted toxicity bioassays [4, 6-10]. In 1982, Curtis, *et al.*, found a linear relationship between Fathead minnows and the Microtox™ assay where the lethal concentration at 50% (LC_{50}) is given by:

$$LC_{50} = 0.80*(EC_{50}) + 0.45 \quad (1)$$

Furthermore, a correlation coefficient of r = 0.83 was found between the two assays [6]. In 1990, Firth and Backman used the Ceriodaphnia Chronic Value (ChV) to relate to Microtox™:

$$ChV = 0.012*(EC_{50})^2 - 0.047*(EC_{50}) + 8.659 \quad (2)$$

A correlation coefficient of r = 0.72 was found between the Microtox™ and *Ceriodaphnia dubia* assays [6].

Polarography

In the polarographic method, the potential of a mercury drop substrate is changed, and the substance of interest is electrochemically reduced at the substrate surface. The magnitude of the reduction current is proportional to the reactant's concentration, and the potential at which the reduction occurs uniquely identifies the substance responsible for the current. The solution in which this process occurs is termed the supporting electrolyte. A more detailed description is given by Bundy, *et al.* [11].

Specifically, differential pulse polarography (DPP) was used in all polarographic measurements. This is due to the fact that DPP minimizes the capacitive effect of the microelectrode charging current and is, therefore, more sensitive than other pulse polarographic techniques [12, 13].This technique uses a waveform that combines a linearly changing voltage with pulses of fixed magnitude superimposed on this potential ramp. The pulses are repeated once during each drop lifetime where the current is measured once before applying the pulse and once during the last few milliseconds of the pulse. The resultant polarogram is, therefore, a plot of current difference versus applied potential. With DPP, the peak current is a quantitative measure of concentration. The potential at which this peak occurs is termed the half-wave potential, $E_{1/2}$. It is unique for every oxidation state of every element—an attribute which makes polarographic methods useful for speciation studies.

PROCEDURES

Aqueous phase measurements

Various heavy metals were tested using the Microtox™ Acute Toxicity Basic Test Procedure in both single element and two-component mixture cases. The reagent (freeze-dried luminescent bacteria), reconstitution solution (doubly distilled water), adjusting solution (22% sodium chloride, NaCl), diluent (2% NaCl), and cuvettes used in the procedure were from Microbics Corporation. The testing instrumentation consists of a Microbics 500 Toxicity Analyzer integrated with an IBM PC PS2/Model 55. The Microtox™ Data Capture and Reporting Program Version 7.82 software was used for acquisition and analysis of data.

Single elements and oxidation states tested in aqueous solution included lead, Pb(II); mercury, Hg(II); cadmium, Cd(II); arsenic, As(III) and As(V); copper, Cu(I) and Cu(II); and chromium, Cr(III) and Cr(VI). All metal species were tested using the Basic Test procedure (aqueous phase) with the exception of Cr(III). Upon pH adjustment, this heavy metal precipitated out of solution necessitating a solid phase test protocol, the specifics of which are described later. All metals were tested at an initial concentration (highest concentration with which bacteria comes in contact) of 45%, with the exception of Cu(I) which underwent a primary dilution of 1:100 to 0.45%. The % refers to the percentage of the initial concentration of the substance before undergoing any series dilutions. Serial dilutions are made to get a range of test concentrations so that the EC_{50} can be determined (see Figure 1).

Each one of the solutions was tested after a pH adjustment (if necessary) through the addition of either hydrochloric acid, HCl (pH > 8), or sodium hydroxide, NaOH (pH < 6), to obtain an optimal pH between 6 and 8. In addition, as part of the standard operational procedure of the Basic Test, a 10% volume/volume of Osmotic Adjusting Solution was added to the sample to obtain a saline concentration of approximately 2% NaCl in order to provide the appropriate osmotic pressure for protection of the bacteria. The toxicity of each sample was determined after 1, 5, and 15 minutes of exposure, with the exception of Cr(VI) which was tested after one minute of exposure only. In future work, dilution of Cr(VI) solutions will be needed due to the fact that longer exposure of the bacteria to Cr(VI) in the tests performed here resulted in a complete extinction of light output of the microorganisms, preventing an EC_{50} calculation.

Besides tests with pure metals, described above, mixtures containing equal volume fractions of either Pb(II) and Cd(II), Pb(II) and Cu(I), or As(V) and Cd(II) were also tested with Microtox™ after 1, 5, and 15 minutes of exposure. All component initial concentrations were 45%, with the exception of Cu(I) which was diluted to a 4.5 % solution. The same procedures as described for the single element cases were followed.

Polarographic techniques were used to measure concentrations and specific oxidation states of heavy metals. The basic methodology was described in the theory section. The specific polarographic measurement equipment used consisted of an EG&G Model 303A Static Mercury Drop Electrode, an EG&G Model 384B Polarographic Analyzer, and a Houston Instruments DMP-40 Digital Plotter.

Single component solutions of Pb(II), Cd(II), Cr(III), Cr(VI), and Cu(II) were tested to

obtain the characteristic half-wave potential ($E_{1/2}$). In the multi-element mode, mixtures containing Cd(II) and Pb(II) in equal volume proportions were tested. The supporting electrolyte used for the mixture test was a 0.1 M tartaric acid adjusted to pH 9 with ammonium hydroxide (NH_4OH). In all cases, DPP was employed.

Complexation study protocol

The complexation of heavy metal ionic forms by organic complexing agents, specifically the soil component humic acid, was studied. The main motivation of this phase of our research is that the toxicity of a metal can be significantly altered by complexation [14]. In addition, greater insight into environmental speciation can be obtained. Also, such measurements can help to avoid artifacts in polarographic measurements caused by complexation.

Two 0.4 mM Pb(II) solutions containing 0.4 mg/ml and 0.8 mg/ml humic acid, respectively, were prepared. Initial Pb(II) concentration was 1,000 ppm in the form of lead nitrate, $Pb(NO_3)_2$, in 2% nitric acid. The mixtures were allowed to soak, undisturbed, for 24 hours to allow complexation to occur. The resultant mixture was analyzed using the Microtox™ Acute Toxicity Basic Test Procedure described above.

Sediment phase measurements

Kaolin clay specimens (from Source Clay Minerals Repository, University of Missouri-Columbia), were soaked in solutions containing Pb(II) or Cr(III) alone at an initial concentration of 1,000 ppm and in a solution containing a 50-50 volume mixture of Pb(II) as $Pb(NO_3)_2$ in 2% nitric acid and Cr(III) as chromium chloride ($CrCl_3$) at concentrations of 500 ppm each. The purpose of using the solution containing the mixture of the two heavy metals was to establish a situation in which the two would competitively sorb onto the clay particles. In all cases, the sediment and soaking solution were stirred using a Cole Parmer magnetic stirrer Model 0639-00 and soaked for 24 hours to allow sorption to occur.

The heavy metal-laden kaolin model sediments were then analyzed using the Microtox™ Acute Toxicity Solid-Phase Test after 10 minutes of exposure. This test allows the microorganisms to come in direct contact with the solid sample in an aqueous suspension of the test sample. This is achieved by combining 35 ml of solid-phase diluent and 7 grams of the solid sample and stirring prior to testing to obtain a more homogeneous sediment sample. The solution is allowed to settle, and the aqueous phase is then filtered out. It is imperative that the temperature throughout the testing period be maintained at 15 degrees Celsius. The reagent (freeze-dried luminescent bacteria), reconstitution solution (double distilled water), solid-phase diluent (2% NaCl), cuvettes, and solid-phase tubes and filters used were again from Microbics Corporation. Instrumentation was the same as for the aqueous measurements.

RESULTS

Aqueous phase measurements

A combination of polarographic techniques and Microtox™ toxicity tests were used to characterize heavy metals of interest. In Table 1, a listing of the half-wave potentials determined for various metals and oxidation states using different supporting electrolytes (S.E.) is shown. The method used was DPP, where the $E_{1/2}$ values were determined with reference to a silver/silver chloride (Ag/AgCl) electrode.

We have developed an analytical technique that allows for simultaneous measurement of the concentration of Pb(II) and Cd(II) in a single supporting electrolyte containing 0.1 M tartaric acid adjusted to pH 9 with ammonium hydroxide. A polarogram for such a mixture is shown in Figure 2. Two distinct peaks can be seen on the polarogram at -0.544 V and -0.732 V corresponding to Pb(II) and Cd(II), respectively. These values are close to those presented in Table 1.

The Microtox™ method was used to determine the EC_{50} of various ions in order to assess their relative toxicity. All metals were tested using the aqueous phase protocol with the exception of Cr(III) for reasons previously mentioned. Table 2 shows the toxicity ranking of heavy metals tested in terms of one minute EC_{50}'s.

The list in Table 2 is ordered from most toxic to least toxic, as determined using the Microtox™ method. The results demonstrate reasonably good agreement with threats posed to human health by these heavy metal chemical forms.

In addition, three aqueous phase mixtures were tested: As(V)/Cd(II), Pb(II)/Cd(II), and Pb(II)/Cu(I). Toxicity results after one minute of exposure are shown in Figure 3.

Complexation experiments

Two mixtures of Pb(II) and humic acid were employed to investigate the interactions of organic complexing agents with heavy metal ions and their effects on toxicity using Microtox™. Results are shown in Table 3. In both mixture cases, for all exposure times (1, 5, and 15 minutes), the chelated mixtures are somewhat less toxic than the solution containing Pb(II) alone. This indicates that the complexed form of Pb(II) is less toxic

TABLE 1. LIST OF SUPPORTING ELECTROLYTES, DETECTION LIMIT THRESHOLDS IN WATER, AND $E_{1/2}$ FOR DIFFERENT PULSE POLAROGRAPHIC ANALYSIS.

Metal species	S.E.	Detection limits (ppb)	$E_{1/2}$, Ag/AgCl (V)
Pb(II)	0.1 M Citric Acid + NH_4OH (pH=3.0)	< 10	- 0.60[a]
Cr(VI)	0.1 M Tartaric Acid + NH_4OH (pH=9.0)	< 10	- 0.84[a]
Cr(III)	0.1 M KCl	100	- 0.99[a]
	5.0 M $CaCl_2$	300	- 1.17[a]
	0.2 M NaSCN (or KSCN) + 0.2 M Acetic Acid (pH=3.2)	30	- 1.02[a] (- 0.96)[b]
Cu(II)	0.1 M Tartaric Acid + NH_4OH (pH=9.0)	< 10	- 0.34
Cd(II)	0.1 M Tartaric Acid + NH_4OH (pH=9.0)	< 10	- 0.74
	0.1 M Citric Acid + NH_4OH (pH=3.0)	< 10	- 0.66

[a]values determined in previous environmental studies [15-17]
[b]value indicates $E_{1/2}$ for Cr(III) in KSCN

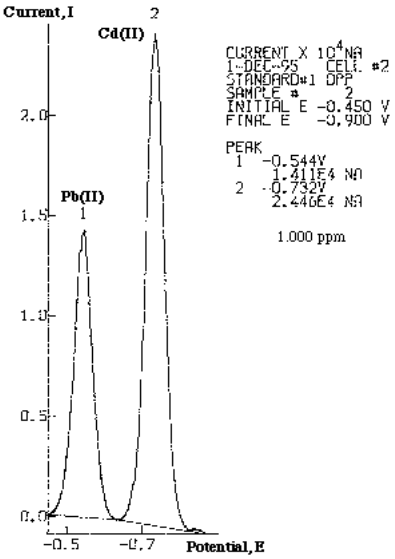

FIGURE 2. SAMPLE POLAROGRAM FOR TWO-COMPONENT MIXTURE.

than the free ionic form.

Sediment phase measurements

Finally, the EC_{50} values for Cr(III)-laden and Pb(II)-laden sediments were determined using the solid phase protocol. Results from both the mixed sediment and competitive sorption cases are shown in Figure 4. Two procedures were performed with the

TABLE 2. RELATIVE TOXICITY RANKING OF VARIOUS IONS FROM MICROTOX™ (ONE MINUTE EXPOSURE TIMES).

Metal species	Average EC_{50} (mg/l)	95% confidence interval
Hg(II)	0.0162	0.0142 - 0.0184
As(V)	1.9548	1.5591 - 2.4508
As(III)	3.1881	2.9820 - 3.4084
Pb(II)	4.1307	3.4361 - 4.9657
Cd(II)	8.7447	7.2574 - 10.537
Cr(VI)	20.3477	19.338 - 21.357
Cr(III)	22.1758	17.309 - 28.412
Cu(II)	626.6674	448.154 - 876.289
Cu(I)	782.7234	626.130 - 978.479

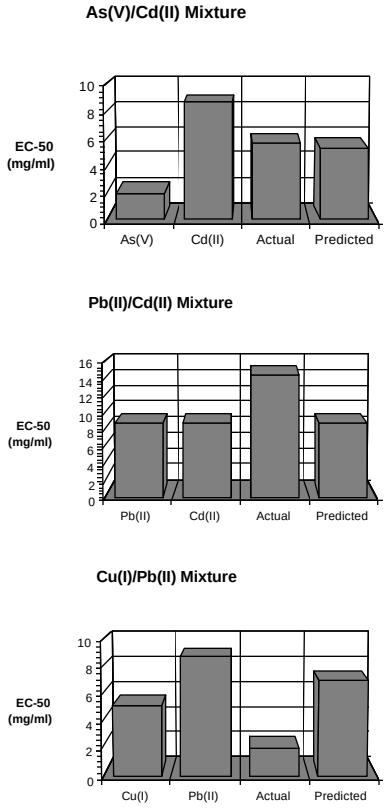

FIGURE 3. PREDICTED AND ACTUAL ONE MINUTE EC_{50} VALUES FOR 50-50 MIXTURES.

sediment phase. The mixed sediment (MS) protocol involved two separate sediment samples soaked in either a Cr(III) or Pb(II) spiking solution. After soaking for 24 hours, the respective spiking solutions were removed through centrifugation. The two resulting sediment samples were placed in a 50-50 volume mixture and tested. For the case of competitive sorption (CS), a single sediment sample was soaked with a 50-50 volume mixture of Cr(III) and Pb(II). Again, the solution was removed after 24 hours and the sediment tested.

DISCUSSION

The polarographic technique is advantageous in that the potential at which the reduction occurs, $E_{1/2}$, is unique to the valence states and chemical forms of the

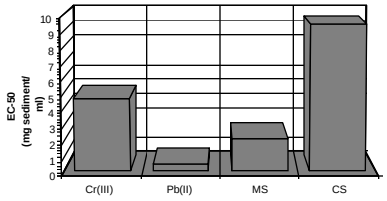

FIGURE 4. SEDIMENT TEST RESULTS FOR SORPTION OF PB(II) AND CR(III) TO KAOLIN.

elements present. Thus, it is possible to determine the specific forms of heavy metals present in a sample by observing the $E_{1/2}$ value, as seen in Table 1. This allows the effects of speciation to be assessed. The concentration of these metal species is directly proportional to the current measured. In this research, we have developed an analytical technique that allows polarographic methods to be used in the multi-element mode. Mixed wastes pose many problems in the environment, and analyses capable of multi-element detection can be of particular use in such situations. Simultaneous measurement of the concentration of Pb(II) and Cd(II) in a single supporting electrolyte containing 0.1 M tartaric acid adjusted to pH 9 with ammonium hydroxide was performed, as seen in Figure 2, and shows good agreement with $E_{1/2}$ values determined for single species. It is feasible that this method may be extended to other elements, provided that a common supporting electrolyte exists.

TABLE 3. HUMIC ACID RESULTS—EC_{50} VALUES (mg/l) AT DIFFERING EXPOSURE TIMES.

Exposure time (min)	1	5	15
Humic acid only	33.7433	35.7723	37.3434
Pb(II) only	4.1307	0.9051	0.1555
Mix 1[a]	6.5735	1.5706	0.6869
Mix 2[b]	5.3654	4.5022	3.2636

[a]Mix 1: 0.4 mM Pb(II) + 0.4 mg/ml humic acid.
[b]Mix 2: 0.4 mM Pb(II) + 0.8 mg/ml humic acid.

The toxicity ranking based on one minute EC_{50} values using Microtox™ demonstrates good agreement with threats posed to human health by these species. As seen in Table 2, of all heavy metal species tested, Hg(II) was found to be the most toxic at 0.0162 mg/l, as would be expected from known health threats, and Cu(I) was the least toxic at 782.7234 mg/l. In addition, speciation effects were exhibited by arsenic, chromium, and copper. As previously mentioned, Cr(VI) was found to be more toxic than the trivalent form. This is as expected since Cr(III) is an essential trace element and has a low order of toxicity whereas the hexavalent state has been implicated in allergic hypersensitivity reactions and carcinogenesis [1]. Cu(II) was significantly more toxic than Cu(I).

In general, inorganic arsenic is more mobile than organic forms and poses more problems regarding leaching into surface waters and ground water. In anaerobic soil the more toxic form, As(III), prevails while the less toxic As(V) is dominant in aerobic soils [18]. However, in this study As(V) was found to be more toxic than As(III). This may be in part due to a poor correlation of toxic responses to arsenic between *V. fischeri* and the human body.

The Environmental Protection Agency (EPA) and Food and Drug Administration (FDA) have set the following limits of heavy metals in drinking water [19-21]:

Cu: 1.3 ppm
Cr: 100 ppb
As: 50 ppb
Cd: 5 ppb
Pb: 5 ppb (limit for bottled water)

Hg: 2 ppb

These follow the general trend of toxicity illustrated in Table 2 where the more toxic species have more stringent limits.

For the As(V)/Cd(II) mixture (see Figure 3), the resultant EC_{50} is consistent with additive behavior, where the predicted value is 5.3498 mg/l and the actual is 5.7176 mg/l. In contrast, the Pb(II)/Cd(II) mixture is about half as toxic as the average of the individual components indicating the presence of antagonistic interactions. The predicted response, based on additive behavior, is 6.078 mg/l whereas the actual value is 14.5985 mg/l. The Pb(II)/Cu(I) mixture, on the other hand, exhibits synergistic behavior to some degree where the resultant mixture is more toxic than would be predicted. Numerically, the actual EC_{50} is 1.9071 mg/l while the predicted value based on additive behavior is 6.9577 mg/l.

Binding of heavy metals by humic acid can occur as chelation between a carboxyl and phenolic hydroxyl group, as chelation between two carboxyl groups, or as complexation with a carboxyl group [12]. In prior studies, our research group has established that complexing agents such as ethylenediamine tetraacetic acid (EDTA) and humic acid will combine with heavy metal ions, making then unavailable for participation in oxidation/reduction reactions [22]. This means that a polarographic measurement, in combination with a total metal content determination (using atomic absorption spectroscopy or inductively coupled plasma atomic emission spectroscopy, for example), can be used to find the relative amounts of free ionic and complexed metal.

Because of their acid-base, sorptive, chelating, and complexing properties, humic substances have strong effects upon the properties of water [12]. For example, humic acid may accumulate large quantities of metals through exchange of species with water. Thus, altering the concentration of humic acid in solution will affect the amount of heavy metal chelated. The degree of this chelation can be qualitatively assessed through examination of the results from our mixture tests. In mix 2 (see Table 3), doubling the concentration of humic acid relative to Pb(II) results in a less toxic sample. After five minutes of exposure, the mixture containing more humic acid is three times less toxic than the less concentrated mix; i.e. it has an EC_{50} value three times higher. After 15 minutes, mix 2 is more than five times less toxic than mix 1. This decrease in toxicity is presumably due to the fact that a larger proportion of Pb(II) is being chelated.

The EC_{50} values for Cr(III)-laden and Pb(II)-laden sediments individually determined using the solid-phase protocol were 4.7369 and 0.4757 mg sediment/ml solvent, respectively. This is consistent with results from the aqueous phase; however, for the solid phase, Pb(II) exhibits a toxicity level ten-fold greater than that of Cr(III) (compared to an approximately 5-fold difference in the aqueous phase—see Table 2). A 50-50 volume mixture of the two combined sediments yields an EC_{50} of approximately 2.1711. If solely additive behavior is assumed, a theoretical EC_{50} value of 2.6063 mg sediment/ml solvent is attained. Thus, as can be seen graphically in Figure 4, a mixed sediment with Pb-laden and Cr-laden fractions in equal proportions has an EC_{50} value consistent with additive behavior.

For the case of competitive sorption, however, the resultant EC_{50} is 9.4738 mg sediment/ml solvent. That is, the EC_{50} value is about twice that for Cr(III) alone,

presumably because preferential adsorption occurs for Cr(III) thereby blocking Pb(II) adsorption to kaolin. The factor of two exhibited by the EC_{50} results from a 50% Cr(III) solution concentration reduction, and therefore a presumed 50% sediment sorbed concentration reduction.

In this work, it is evident that the combination of polarographic and Microtox™ techniques is quite useful for analysis of complex mixtures of pollutants. Polarography is beneficial not only in determining the effects of speciation, but also can be used in both the single and multi-element mode, making it useful for analysis of complex heavy metal mixtures. Microtox™ clearly illustrates the effects of synergistic and antagonistic interactions between species; therefore it is possible to determine whether the toxicity of a mixture is more or less toxic than the sum of its parts. When used in conjunction, these methods are capable of accurately assessing and quantifying the toxicity of various heavy metal mixtures.

Thus far, the polarographic and Microtox™ techniques have been applied only in the laboratory setting. In many situations involving environmental samples, it would be better if analysis could be performed in the field directly, however. Besides allowing analytical results to be obtained more rapidly on site, direct field sensing can be useful for coping with very inhomogeneous distributions of pollutants that are often seen in the environment. Furthermore, by eliminating the trip to the laboratory, it may be possible to reduce the conversion of certain ions to other chemical forms.

Despite a clear need for a bioluminescent bacterial toxicity test that is field deployable, there have been only limited attempts to use Microtox™ methods directly in the field. Such an extension of this method seems quite feasible, however. A field polarographic sensor is presently under development [11]. In addition, Young, *et al.*, described a portable custom electronic circuit of their devising that could perform polarographic nitrate analyses [23]. It is therefore feasible that a combination polarographic and Microtox™ assay method could be developed for testing under simulated field conditions. This field sensor must be capable of measuring heavy metals in situations where there are mixtures of metals (often in association with various organics) in water, soils, and sediments. In another research project being conducted in our laboratory, this combined technology is currently being investigated.

CONCLUSIONS

Mixed wastes pose a variety of complex questions to environmental science. This research project encompasses an intensive effort to develop methods and technologies that will help us to understand the chemistry and toxicity of water, sediments, and soils contaminated by mixed wastes. The main experimental methods used for this work involve polarography and a Microtox™ analyzer.

Polarography is an electroanalytical technique capable of ppb concentration detection sensitivity. This method can be used in speciation studies since it can identify different metal oxidation states, and in certain circumstances can differentiate between ionically dissolved and complexed metal forms. Furthermore, polarographic techniques are useful in both single element and multi-element modes to identify specific oxidation states of various heavy metals present in complex mixtures.

The Microtox™ analyzer uses a sensor based upon a bioluminescent bacterial assay. The organism involved is *Vibrio fischeri*, a nonpathogenic marine bacterium. The light output of the bacteria is attenuated in the presence of pollutants, and so it is a quantitative measure of contaminant toxicity in water, soils, and sediments. Toxicity measurements of aqueous solutions using Microtox™ demonstrate good agreement with threats posed to human health by the heavy metal chemical forms tested. In addition, synergistic and antagonistic interactions in mixtures have been observed, indicating that some mixtures are more toxic than would be predicted based solely on toxicity values for the individual components. Sediment phase measurements indicate that soils exposed to various toxicants may behave in an additive fashion. Also, competitive sorption may dramatically change the sediment's toxic effects. Finally, Microtox™ methodology allows for the study of interactions of organic complexing agents, such as humic acid, with heavy metal ions to determine the effect of complexation on toxicity.

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