HEAVY METAL SPECIATION AND UPTAKE IN CRAYFISH AND TADPOLES

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ABSTRACT Developing valid pollution recording methods is central to assessing environmental damage and remediation. This often is difficult, however, because of speciation and multiphase distribution of contaminants. Polarography, an electroanalytical technique capable of detection and quantification of trace levels of elements and ionic complexes, is a promising method for analyzing environmental samples. Here, polarography has been used to determine lead concentration in water, sediment, bullfrogs, tadpoles, and adsorbed onto kaolin. It has also been used to measure hexavalent chromium concentration in crayfish. This research involves field studies and two laboratory experiments. Studies of a Louisiana swamp have shown lead's affinity for sediment and water particulate phases, rather than being ionically dissolved in the aqueous phase. In swamp bullfrogs, lead was found in greater concentrations in bone compared to muscle. In the first laboratory experiment, lead uptake originating from water and sediment increased in tadpoles as exposure time and concentration increased. Also, this animal's development was hindered at higher concentrations. The second laboratory experiment exposed crayfish to aqueous hexavalent chromium. Total chromium uptake increased with exposure time and concentration. The chromium tissue abundance was hepatopancreas > gills > muscle. A substantial portion of tissue hexavalent chromium converted to the less toxic trivalent form.

KEYWORDS: speciation, polarography, chromium, lead, crayfish

INTRODUCTION

Environmental pollution is widespread in many industrialized countries and results in contamination of the land, water, and air with which all living organisms interact. In many cases this interaction proves to be toxic to the organisms. Central to characterizing the extent of pollution and toxicity is developing better methods to detect and quantify it. Polarographic methods have shown their suitability for environmental contaminant detection in water, soil, and biota under specific conditions and have been used here.

Polarography is a trace level electroanalytical technique developed by J. Heyrovsky [1], who was awarded the Nobel Prize in chemistry in 1959 for this achievement. In this technique a potential is applied to a drop of mercury suspended in a solution known as the supporting electrolyte. As a range of potential is scanned, the current, due to reduction or oxidation reactions, is measured. As a species starts to react at a given voltage, the current, I, will suddenly increase, over a small potential range, until it reaches a limiting current, I_L. At $I = I_L$, the rate of reaction at the electrode is dependent upon the diffusional transport of the species to the electrode. The potential where the current is one-half the limiting current is defined as the half-wave potential, $E_{1/2}$, a quantity unique to each chemical species capable of reduction. Since the reduction reactions occur under diffusion-controlled conditions in a polarographic cell, I_L is proportional to the concentration of the species present within it. Therefore, polarography may qualitatively identify (by

$E_{1/2}$) and quantify (by I_L) how much of a given species is present. A special solution known as a supporting electrolyte is used in the polarographic cell to minimize electrical migration of the species of interest and to increase the conductivity of the solution [2]. In addition, the solution is purged with pure nitrogen gas to drive off dissolved oxygen, a ubiquitous electroactive contaminant which interferes with measurements. A diagrammatic representation of the method has been given in another paper by our research group appearing in this proceedings volume [3].

The polarographic method can generally detect concentrations as low as 1 ppb and up to 100 to 200 ppm at the high end. However, limitations of polarography include the possibility of interferences from other substances which are reduced at potentials near the potential of the ion of interest, adsorption of some substances onto the electrode creating measurement artifacts, the fact that it is not a multi-element method (in the sense that ICP or EDAX are), and the relatively lengthy time required to analyze many samples.

Once a metal contaminant is introduced into the environment, many possibilities exist as to its chemical form. In natural waters, metals may be found as free ions or as various complexes with both inorganic and organic ligands [4]. For example, over a range of pH and potential values, up to 12 solid or dissolved chemical forms exist for arsenic, 10 for silver and selenium, and 15 for chromium when these metals are dissolved in pure water alone [5]. In soils and sediment, metal ions may be, as well, adsorbed onto the surfaces of minerals and complexed with organic constituents. Alternatively, metal may be retained by soil and sediment if a secondary mineral phase precipitates due to a high concentration of soluble metal in solution [4].

Similarly, many chemicals are found in different forms within living organisms, at least transiently. For example, the two forms of arsenic, As^{+3} and As^{+5}, are methylated to monomethylarsonic acid and dimethylarsinic acid, respectively [6]. Also, chromium may exist as Cr^{+6} or Cr^{+3}. Chromium is able to cross cell membranes and is well absorbed by inhalation or orally in the hexavalent form, but as trivalent chromium it is poorly absorbed and less likely to cross cell membranes.

As is evident, the speciation of metals in the environment and in biological samples is often complex and difficult to ascertain. However, polarography may help to alleviate this difficulty since it is able to determine specific chemical forms of an element. This is of primary importance since the toxicity of an element often varies widely depending upon its form. For example, Cr^{+6} is a known carcinogen, whereas exposure to Cr^{+3} is less harmful [7]. Additionally, the two forms of arsenic listed above differ in toxicity.

Not only do chemicals differ in their chemical form once inside an organism, but they also differ as to what physiological system they influence and where they are sequestered. For example, by weight, ninety percent of the human body's burden of lead is stored in the skeleton [8, 9], and fifty percent of the cadmium burden is found in the kidneys [6].

A study by Hernandez, *et al.* [10], indicated that for crayfish exposed to hexavalent Cr, Atomic Absorption Spectroscopy (AAS) analysis showed the largest amounts accumulated in gill and antennal gland tissue, followed by the hepatopancreas. The

lowest amounts of Cr was found in the abdominal muscle. Also, a study by Anderson and Brower [11] indicated that AAS analysis of crayfish tissues from animals collected from three sites on the Fox River in Illinois showed that the abundance of heavy metals in the environment was: Cd < Cu < Pb < Zn, but in the animal it was Cd < Pb < Cu < Zn. Gills and viscera accumulated the highest levels for all metals except lead. Lead was at the greatest concentration in the exoskeleton. Muscle tissue showed the least uptake of these metals. Although several methods are available to discern the concentration of heavy metals in biological tissues such as those listed above, polarography remains a prudent choice of analytical technique if speciation studies are desired.

OVERVIEW OF STUDIES CONDUCTED

Louisiana contains several contaminated waterways, among them Devil's Swamp and Bayou Trepagnier, the sites which form the basis of much of the research described in this paper. Devil's Swamp is an area adjacent to the Mississippi River near Baton Rouge, LA. Bayou Trepagnier, an area with high concentrations of heavy metals located in St. Charles Parish, LA, is adjacent to a large petrochemical facility and feeds directly into Lake Ponchartrain. In order to fully understand the amount of pollution in the waterways, field studies which analyze contamination in the different components of the environment, such as water, sediment, and wildlife, are needed. However, field studies do not address many of the processes which contribute to the pollution, nor how that pollution affects other components of the environment. Laboratory studies, although isolated from the environment, are able to study those processes in a controlled manner in order to understand the underlying mechanisms. Therefore, a combination of field and laboratory studies, as was performed in this research, should, in principle, provide a more complete picture of a particular environment.

The first phase of this research was a field study involving polarographic detection of lead in Devil's Swamp water, sediment, and bullfrogs. The particulate matter in the water and sediment was separated into various size fractions to determine whether lead is more likely to be found not only in solid versus aqueous components but also coarse versus fine particulate matter. Several bullfrogs, *Rana catesbeiana*, obtained from Devil's Swamp were also polarographically analyzed to determine whether any lead from the environment concentrated in this animal.

The second phase of this research included two laboratory studies involving controlled heavy metal exposure to organisms. Based upon the lead concentration in Devil's Swamp, a laboratory model exploring the uptake of lead and its effect on the development of *Xenopus laevis* tadpoles was conducted. The tadpoles were subjected to lead arising from either the water (ionically dissolved) or a model sediment, kaolin clay. The amount of lead in the model sediment was determined using sorption curves measured in our laboratory. The second controlled laboratory exposure study investigated how hexavalent chromium is absorbed, stored, and excreted from the red swamp crayfish, *Procambarus clarkii*. Crayfish were chosen as subjects because they are not only a popular food source in Louisiana, but are also readily available most of the year and require only simple living conditions for their maintenance. Crayfish gill, hepatopancreas, and abdominal muscle tissues were chosen for analysis in this study.

METHODOLOGY

Polarography

Polarographic measurements were conducted using an EG&G Princeton Applied Research Corporation (PARC) Model 384B Polarographic Analyzer with an EG&G PARC Model 303A Static Mercury Drop Electrode. The form of polarography used in the polarographic experiments was differential pulse polarography (DPP), a dropping mercury method. In this technique, an electrical potential in the form of a staircase ramp is applied to a series of mercury drops which fall off a column of mercury at constant time intervals. Modulation pulses with identical offset voltages are superimposed on the ramp every time just before a drop is dislodged [12]. The current is measured just before the pulse is applied and just before the pulse ends. The currents are compared, with the change in current being the signal to be processed. This method produces a current peak (proportional to the concentration being measured) at $E_{1/2}$. The fact that a new mercury drop is used for each potential increment helps reproducibility of results by minimizing contamination of the electrode over time, particularly by residual organic material. Calibration curves were obtained using the standard addition method where known concentrations of the species of interest are added to the sample containing the unknown.

For diffusion-controlled conditions to exist in the polarographic cell, ions other than those being tested must carry the electrical migration current. Therefore, one adds a supporting electrolyte to the sample in the cell. Careful selection of the supporting electrolyte is needed, in addition to effective separation and extraction methods, for a successful polarographic measurement on an environmental sample. For different supporting electrolytes, the detection threshold may vary substantially. In our experience, best results are obtained for hexavalent chromium and lead polarographic detection when 0.1 M tartaric acid (with ammonium hydroxide added to pH 9) and 0.1 M citric acid (with ammonium hydroxide added to pH 3) are used, respectively. The half-wave potential is dependent upon the supporting electrolyte used, although each species still possesses a unique half-wave potential in a given supporting electrolyte.

Devil's Swamp water, sediment, and bullfrog field study

In this research, polarography was used to quantify the extent of lead contamination in water, sediment, and bullfrogs obtained from Devil's Swamp. The lead concentration was found in various size fractions of the water particulate and sediment, in addition to the aqueous phase of the sampled water. A vacuum filtration system (Vacu/Trol Lab Vac Regulator with Pump by Spectrum Medical Industries, Inc.) was used for this separation. The different size fractions of the solid phases were >290, 105-290, 20-105, and 1-20 μm. In this study, anything less than 1 μm was considered to be the aqueous phase. The aqueous phase was analyzed after boiling to concentrate the metal in the water. For the sediment, the aqueous portion refers to the pore water plus the water added when the sediment was suspended in distilled water and passed through a 1 μm filter. Extraction of lead from the solid phase was performed using acid digestion according to ASTM Standard D 3974 Partial Extraction of Trace Elements from Sediments [13]. The procedure is as follows: heat the sample (4 grams) + 100 ml H_2O, 10 ml 37% HCl, and 1 ml 70% HNO_3 at 95ºC until 10-15 ml of solution remains, which is then able to be polarographically analyzed.

For the bullfrogs, bone and muscle were analyzed for lead to determine whether it is preferentially concentrated in one of the tissues. The extraction of Pb was accomplished by ashing the tissue in a furnace (Heavy Duty Electric Co., Type 051-PT). The muscle was ashed for ½ to 1 hour at 750-800^{o}C prior to polarographic analysis, and the ash was put directly into the polarographic cell. Bone, after ashing for ¾ to 1 hour at 750-800^{o}C, was digested using ASTM D 3974 to dissolve the bone residue to aid in polarographic analysis. To compare results, a few samples were analyzed using Atomic Absorption Spectrometry (AAS, Perkin Elmer 4100 ZL) or Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES, Perkin Elmer Optima 3000) in which case a dilute nitric acid carrier was used. Once the data was collected, the metal concentration in the bullfrog muscle was compared to immunological biomarkers, PHA and ConA, measured by Dr. Elliot Horner of the Department of Immunology of Tulane Medical Center, to see if a correlation exists. PHA, or phytohemagglutinin, and ConA, or concanavalin A, are both proteins that agglutinate mammalian erythrocytes and stimulate T lymphocytes [14].

Lead uptake by tadpoles

Before beginning the tadpole exposure study, a method to alter the lead content of the model sediment, kaolin, was needed. This was accomplished following ASTM D 4646-87 "Standard test method for 24-h batch-type measurement of contaminant sorption by soils and sediments" [15] as a guide. The sorption curve measurement was performed by placing a model sediment in solutions with various concentrations of lead, as lead nitrate, (1 gram sediment:20 ml solution) for 24 hours. Subsequently, the solutions were filtered to retain the sediment, which was then digested and analyzed using differential pulse polarography. Digestion followed ASTM D 3974 to extract Pb^{+2}. The model sediment was the commercially-obtained clay, kaolin (Source Clay Mineral Repository, University of Missouri-Columbia), to ensure a relatively pure substrate.

Based upon the concentration of lead found in the water (3.7 ppb ionically dissolved) and sediment (18.7 ppm) of Devil's Swamp, a controlled laboratory study exploring metal (Pb) uptake from water and sediment by *Xenopus laevis* tadpoles was conducted. This organism was chosen due to its availability, ease of care, genetic consistency, and development to adulthood within 7 weeks. Spiked kaolin, as described above, served as the sediment. The water used in the experiments was tap water filtered through an activated carbon bed to remove chlorine. The water and sediment were replaced every other day, also at which time the tadpoles were fed powdered nettle leaf suspended in water. Lighting was set for a 12 hour bright/12 hour dark cycle. The basic protocol for the experiments is shown in Table 1 where Y represents the number of live animals at the end of the exposure period. During the course of the experiment, a normal amount of attrition resulted, decreasing the original starting number of 8-10 tadpoles per exposure condition to a minimum of 4 tadpoles in some cases. For 5 weeks, tadpoles were exposed to water and sediment containing 1, 5, and 10 times the concentration of lead found in the Devil's Swamp water and sediment samples. Additionally, organisms were exposed to the 5X conditions for 3 and 6 weeks. At the end of the exposure period, the tadpoles were weighed and their developmental stage, as described by Nieuwkoop and Faber [16], was determined. After sacrificing the tadpoles, the lead was extracted from the

animals prior to polarographic analysis. This was accomplished by ashing the tadpoles for ½ to 1 hour and dissolving the ash in 0.1 M citric acid plus NH_4OH to pH 3. This study helped in understanding both the concentration and time effects of pollutant uptake by tadpoles. Additionally, the contribution of sediment vs. water, with regard to which plays a more important role in bioaccumulation, was deduced by comparing results from the controls.

Chromium uptake by crayfish

The scientific literature does not cite a standard method for chromium extraction from tissues. Several methods for extraction of metals from soils and sediments exist, however (including EPA 3060, ASTM D-3974, D-4638-1, and D-4638-2). These methods were chosen as a starting point for polarographic experimental protocol development for crayfish tissue analysis. They were either discarded or adapted depending on the results found when using them. A tissue digestion method (used with success in previous biomaterial studies in our laboratory), that uses sulfuric acid and hydrogen peroxide, was also studied. An intensive investigation program studying these methods was conducted to determine % recovery, concentration detection limit, and % conversion of trivalent to hexavalent chromium associated with them. This effort showed that the optimal procedure to extract hexavalent chromium from the crayfish depended upon which tissue was being analyzed. The following procedures list those extractions found to be most suitable and that were used in this work:

Gill tissue

The digestion method involved adding 2.5 ml H_2SO_4 to the sample, which had been ashed for 45 minutes at 750-800^oC, and heating in a boiling water bath for 2 hours. The solution was then cooled and 4 ml of 30% H_2O_2 was added. The solution was again heated for 2 hours in the water bath. It was necessary to allow the solution to sit at room temperature for 24 hours before testing. The percent recovery, detection limit, and percent conversion for this method was 20-25%, 8 ppb, and 2-3%, respectively.

Hepatopancreas tissue

To prepare these tissues, ashing was found to be unsuitable, and the tissues were dried by heating on a hot plate at 60^oC for 25-30 minutes. The digestion method was as above, except that heating periods were 3 hours and $KMnO_4$ was used as the oxidizing agent. The resulting solutions after digestion

TABLE 1. HEAVY METAL (Pb) LABORATORY EXPOSURE OF *XENOPUS LAEVIS* TADPOLES.

	CONCENTRATION in sediment and water (multiples of that found in Devil's Swamp)		
Time	1X	5X	10X
3 weeks		Y	
5 weeks	Y	Y	Y
6 weeks		Y	

Y>=4

Controls: A. 5 & 6 weeks—unspiked sediment, unspiked water
B. 5 weeks—no sediment, spiked water (1X)
C. 6 weeks—no sediment, unspiked water

appeared to be viscous and dark brown. Recovery was established at 35% and conversion was approximately 15%. Of the three examined, the detection threshold was the highest for this tissue at 21 ppm in the tissue.

Muscle tissue

For muscle tissue samples, it was necessary to increase ashing time in the furnace to 65-70 minutes at 750-800^{o}C. The digestion method was the same as that for the hepatopancreas given above. Heating times for samples totaled 6 hours at which point a viscous, dark brown solution resulted. This method showed 30-35% recovery, 15% conversion of trivalent chromium to hexavalent, and had a detection limit of 680 ppb.

No method was found to be successful for polarographically measuring Cr^{+3}. Thus, AAS was chosen to measure total chromium concentration, and the trivalent chromium concentration was then determined by difference. AAS measurements were conducted using a Perkin-Elmer Model 5200 ZL atomic absorption spectrophotometer with Zeeman background correction and a graphite furnace with autosampler. High purity argon gas was used.

The selection of which crayfish organs would be tested was made using the following rationale. Gills were chosen as an organ having direct contact with the toxic chromium. The hepatopancreas was chosen as an organ that processes chemicals and detoxifies them much in the way that the human liver does. Finally, the abdominal muscle was studied as an organ of possible storage for chromium in the crayfish body and since it is an important food source.

The exposure details were as follows. Only females with carapace length measuring between 20 and 48 mm were used. Approximately 30 animals were placed in each of four tanks with 4 l of room temperature tap water. Lighting was controlled by use of timers set for 12 hours light and 12 hours dark. The crayfish were fed commercial crayfish chow (People's Moss Gin Co., Milwaukee, WI) 4 times weekly, at least 1 hour prior to changing tank water. The tissues were weighed and kept frozen at -10^{o}C until analyzed. After 2 weeks purging, the crayfish were exposed for periods up to 7 weeks in water with concentrations of 0.3, 3.0, and 30.0 mg Cr^{+6}/l (in the form of potassium dichromate). Animals were sacrificed at time 0 (control), 4, and 7 weeks exposure time.

RESULTS AND DISCUSSION

Devil's Swamp water and sediment analysis

Devil's Swamp, an area near Baton Rouge which contains a Superfund site, was chosen as the field site to test the suitability of polarography in analyzing Louisiana environmental samples. Figures 1 and 2 show the concentration of lead in various moieties of Devil's Swamp water and sediment samples. As expected [17], lead is found in higher concentrations in the sediment as compared to the water. The overall lead concentration in Devil's Swamp water was only 14.71 ppb or roughly 1000X less than the concentration in the sediment (18.7 ppm). Also, only 25%, or 3.67 ppb, of the lead found in the water was dissolved; the rest was associated with particulates. Similarly, the aqueous portion of the sediment contained only 5.5 ppb Pb compared to 18.7 ppm Pb for the solid components of the sediment. The lead concentration in the different size fractions of Devil's Swamp sediment differed by, at the most, about 3.5-fold, indicating some

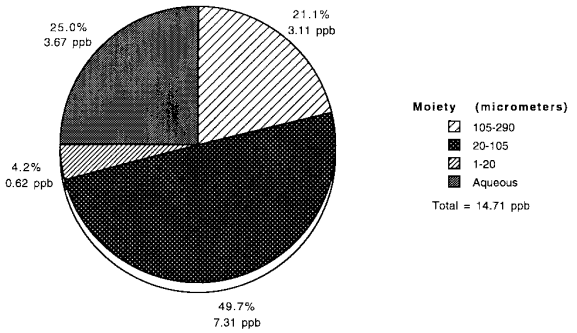

FIGURE 1. PARTITIONING OF LEAD IN DEVIL'S SWAMP WATER (ppb = μg Pb IN MOIETY/l WATER).

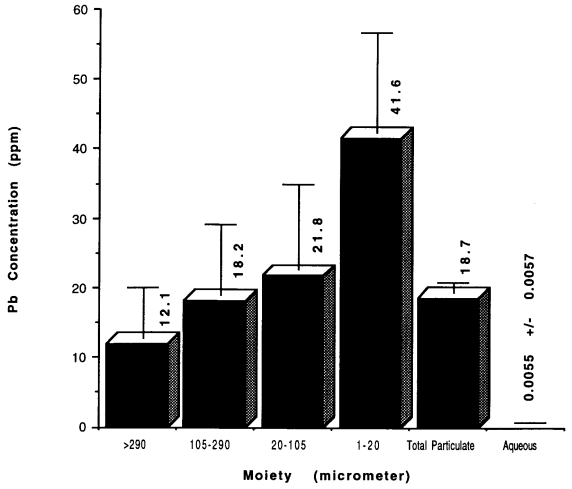

FIGURE 2. LEAD CONCENTRATION IN VARIOUS COMPARTMENTS OF DEVIL'S SWAMP SEDIMENT.

uniformity in the lead distribution in the sediment. The greatest lead concentration was in the 1-20 μm moiety, roughly the range in which many clays fall [18]. This is consistent with lead's affinity for sediment and clays in particular.

Devil's Swamp bullfrogs analysis

In addition to water and sediment, bullfrogs (*Rana catesbeiana*) from Devil's Swamp were collected to determine the lead and chromium content in bone and muscle. Figure 3 shows the Pb and Cr concentration

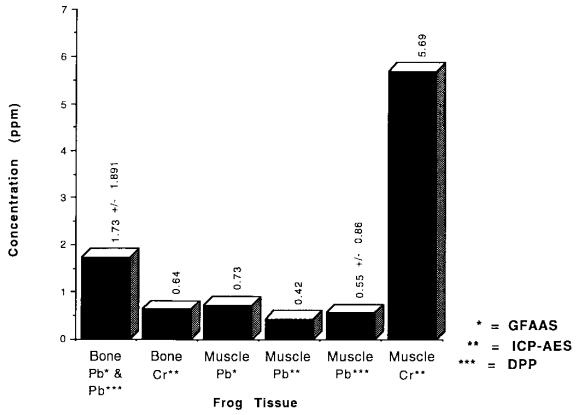

FIGURE 3. CONCENTRATION OF LEAD AND CHROMIUM FOUND IN BULLFROGS FROM DEVIL'S SWAMP.

in these tissues. AAS and ICP-AES were used to compare results to differential pulse polarography. For each column in Figure 3, the number of samples analyzed using DPP, AAS, and ICP-AES were 8, 1, and 1, respectively. Although the number of measurements is limited, comparison of bullfrog muscle lead content for all three methods does not reveal any important difference between them. As for the bullfrog tissues, lead appears to accumulate in bone more than muscle, as expected. For both tissues it is present in amounts much larger than the concentration in the swamp water. Alternatively, chromium accumulates preferentially in muscle compared to bone. The concentration of lead in bone was greater than the chromium concentration. However, muscle was more enriched with chromium than lead.

Figures 4 and 5 compare the lead concentration in muscle to two immunological biomarkers, PHA and ConA, as pointed out earlier, two proteins that agglutinate mammalian erythrocytes and stimulate T lymphocytes. As seen in Figures 4 and 5, a correlation between lead muscle concentration and PHA and ConA

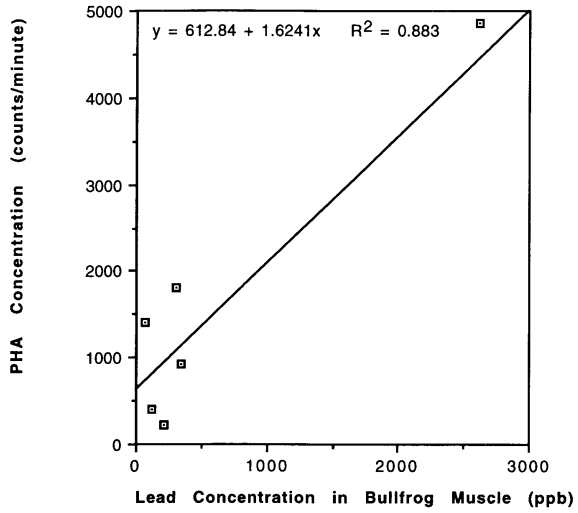

FIGURE 4. SPLEEN LYMPHOCYTE FUNCTION INDICATOR, PHA VS. POLAROGRAPHIC MUSCLE LEAD CONCENTRATION IN DEVIL'S SWAMP BULLFROGS.

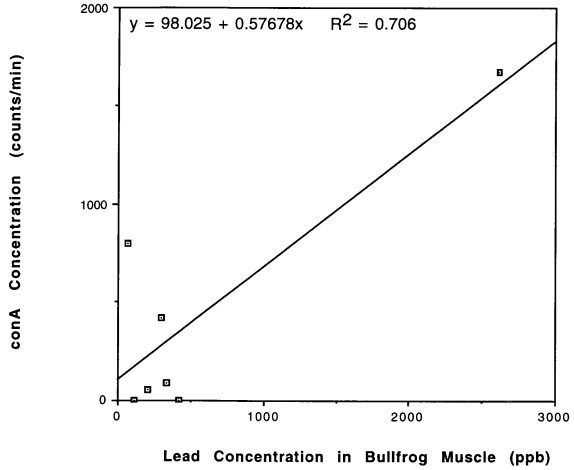

FIGURE 5. SPLEEN LYMPHOCYTE FUNCTION INDICATOR, ConA VS. POLAROGRAPHIC MUSCLE LEAD CONCENTRATION IN DEVIL'S SWAMP BULLFROGS.

concentration may exist, although one cannot be sure about this due to the uneven spacing of the data points. A possible explanation for the increase in PHA and ConA with Pb concentration follows. When challenged by Pb accumulation, the body needs to excrete the lead or render it in a benign form. Production of white blood cells (lymphocytes) and proteins, i.e., PHA and ConA, which stimulate that production, may be mechanisms designed to reduce the lead concentration in the body. Another interesting hypothesis based upon this correlation is related to the fact that lead toxicity is associated with anemia. An increase in proteins which cause red blood cells to clump together in response to an increase in lead concentration could conceivably cause this condition.

Tadpole laboratory exposure to lead

Based upon the field data from Devil's Swamp presented above, a laboratory study examining the metal uptake by tadpoles was conducted. For a period of 3, 5, and 6 weeks, tadpoles were subjected to 1, 5, and 10X the concentration of lead found in Devil's Swamp water and sediment, 3.7 ppb and 18.7 ppm, respectively. The sorption curve for Pb^{+2} in kaolin is given in Figure 6. The sorption curve was then used as a basis for loading sediment with lead in the exposure study.

At the conclusion of the experiment, the tadpole's body weight, developmental stage, and whole body lead concentration were determined. Figure 7 shows the effect of lead exposure level on lead uptake at 5 weeks. Not surprisingly, the Pb uptake increased with lead exposure concentration, although the increase was not in proportion to the increase in exposure concentration. The tadpoles exposed to the 1X level and the controls did not significantly differ, indicating the tadpoles retained lead originating from both the water and sediment since Control A only contained lead naturally in the kaolin (roughly 13 ppm) and Control B only contained lead in the water at the 1X (3.67 ppb) level. This assumes no lead is present in the tap water or food.

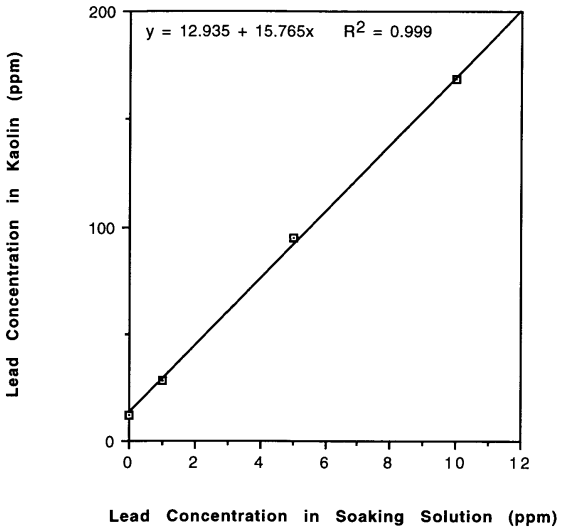

FIGURE 6. LEAD SORPTION CURVE FOR KAOLIN.

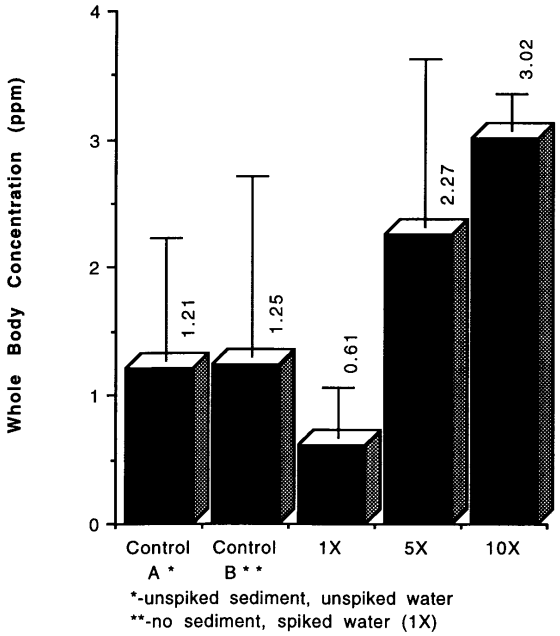

FIGURE 7. INFLUENCE OF Pb EXPOSURE LEVEL ON Pb UPTAKE BY *XENOPUS LAEVIS* TADPOLES AT 5 WEEKS.

However, it is likely very trace levels of lead are present from both of these sources, but that this lead is not taken up by the tadpoles at levels significant enough to explain the results. This explanation is plausible since a third control, in which the only lead present would originate from the tap water or food, had 2 tadpoles at 1 ppm whole body concentration and 3 tadpoles with levels below the polarographic detection limit (corresponding to approximately 200 ppb whole body lead concentration).

For tadpoles exposed to the same lead concentration, the 5X level, the uptake of lead increased with time. For 3, 5, and 6 weeks, the lead concentration in the tadpoles was 2.16, 2.27, and 5.11 mg Pb/kg whole body weight, respectively. Thus, for the first five weeks, growth keeps pace with lead uptake to maintain a roughly constant concentration. As the growth slows down in the latter stages of development, whole body lead concentration of the tadpole increases. Figure 8 illustrates the effect of lead exposure upon body weight at 5 weeks. As exposure level of lead increases, body weight decreases. Similarly, as exposure level of lead increases, tadpole physical developmental, as judged by the stages described by Nieuwkoop and Faber [16], suffers. The developmental stages of *Xenopus laevis* tadpoles at 5 weeks for Control A, Control B, 1X, 5X, and 10X exposure levels were 60-64, 59-61, 61-64, 56-60, and 51-54, respectively. Thus, as with humans, lead hinders the proper development of tadpoles from juvenile status to adulthood.

Crayfish laboratory exposure to hexavalent chromium

Results from the crayfish chromium exposure show that total Cr content in gill and hepatopancreas tissues, but not in muscle, generally increases with elevated Cr^{+6} concentrations in water as shown in Figure 9. However, this is not evident for hexavalent Cr (see Figure 10). For all tissues

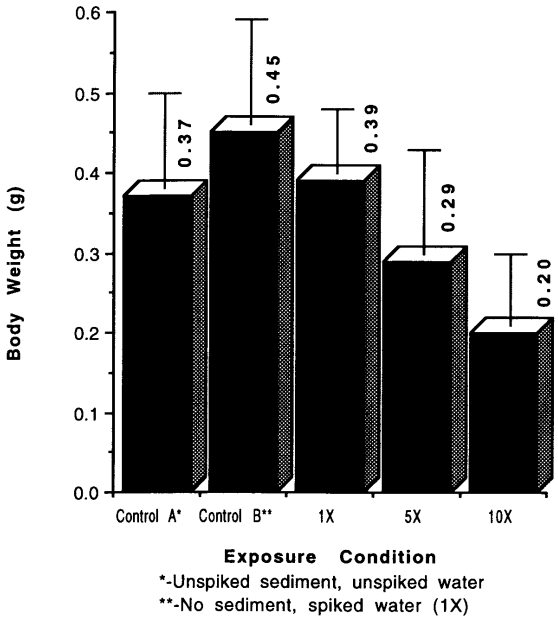

FIGURE 8. INFLUENCE OF Pb EXPOSURE LEVEL ON BODY WEIGHT OF *XENOPUS LAEVIS* TADPOLES AT 5 WEEKS.

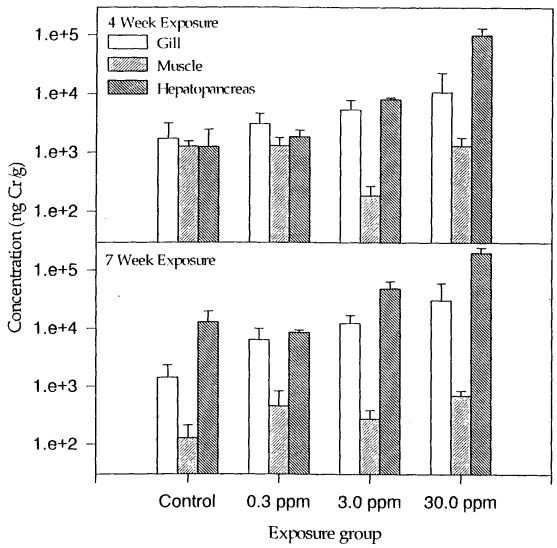

FIGURE 9. COMPARISON OF TOTAL CHROMIUM CONCENTRATIONS DETERMINED BY AAS IN CRAYFISH GILL, MUSCLE, AND HEPATOPANCREAS TISSUES AFTER 4 AND 7 WEEKS EXPOSURE TIME. NOTE: IN THE GRAPHS ABOVE, DIFFERENT CONCENTRATION SCALES WERE USED FOR CLARITY OF THE FIGURES.

and exposure concentration groups (except for the muscle at four weeks), total Cr content generally exceeds control values at a given exposure concentration. Total Cr content increased with time for gills and hepatopancreas but decreased in muscle. For hexavalent chromium on the other hand, only for the hepatopancreas was the concentration greater than the control level for the majority of the exposure time/water concentration combinations. None of the tissues generally showed a statistically significant increase in Cr^{+6} concentration with time at a given water concentration.

Not surprisingly, the concentration of chromium in the different crayfish organs was not equal. The typical tissue concentration ranking for total Cr and Cr^{+6} for 4 and 7 week exposures for all concentration groups was hepatopancreas > gill > muscle tissue. Furthermore, some organs appear to clear the chromium, whereas in others the half-life of chromium in the tissues is longer. This is evident because control groups demonstrate Cr clearance in the muscle and hepatopancreas, but not in the gills. Another interesting result shows that in a majority of time/exposure group combinations, total Cr > Cr^{+6}, indicating a mechanism may exist in these tissues to convert toxic Cr^{+6} to the less toxic Cr^{+3} form. This was particularly true at the 3 and 30 ppm exposure concentration. For example, for these concentrations the degree of conversion observed ranged from 53 to 95% for gill tissue and 47 to >89% for the hepatopancreas.

CONCLUSIONS

Field studies indicated lead has a higher affinity for solid phases such as sediment and water particulates than for aqueous

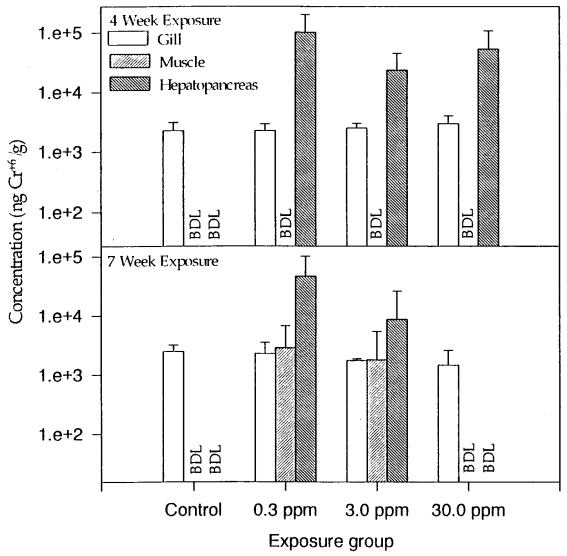

FIGURE 10. COMPARISON OF HEXAVALENT CHROMIUM CONCENTRATIONS DETERMINED BY POLAROGRAPHY IN CRAYFISH GILL, MUSCLE, AND HEPATOPANCREAS TISSUES AFTER 4 AND 7 WEEKS EXPOSURE TIME. NOTE: IN THE GRAPHS ABOVE, DIFFERENT CONCENTRATION SCALES WERE USED FOR CLARITY OF THE FIGURES; "BDL" MEANS BELOW DETECTABLE LIMITS.

phases. This is further supported by our measured sorption curve for lead which shows large increases in the amount of lead adsorbed onto kaolin when the lead concentration in the soaking solution increases. Further field studies involving bullfrogs showed the lead concentration in bone to be greater than that in muscle, and the chromium concentration in muscle to be greater than that in bone.

A laboratory experiment exploring the uptake of lead in *Xenopus laevis* tadpoles, originating from both the water and sediment, found that the animals sequestered more lead as time and exposure concentration increased. However, the development of the tadpoles, as measured by body weight and stages status, was hindered as exposure concentration increased. A second laboratory experiment, exposure of crayfish to hexavalent chromium, found that, in general, total chromium increased as exposure time and concentration increased. Also, the chromium was found in the greatest amount in the hepatopancreas, secondly in the gills, and at the least concentration in the muscle tissue. Overall, successful methods were developed for Cr speciation and uptake studies in various crayfish tissues. Evidence of conversion of hexavalent Cr to the less toxic Cr^{+3} form was found for all tissues.

Polarography has various potential advantages as a technique for detection of pollutants. Determining the chemical form of an element is central to assessing its toxicity. Polarography is able to detect different valence states, as was used in this research for chromium. Additionally, as is evident from the results, low concentration thresholds are attainable. Therefore, polarography remains a valuable analytical technique when environmental speciation studies are desired.

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