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Bioavailability of lead from vitrified slagged aggregate

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Abstract

This study was conducted to determine the leachability of lead (Pb) from a vitrified slagged aggregate (VSA) into water, as well as the bioaccumulation of lead into specified tissues of sunfish (*Lepomis megalotis*) maintained in aquaria containing VSA over a period of 100 days. The experiments consisted of solubility studies comparing the leaching or release of lead from 600 and 1200 g of VSA into aquaria. Over the course of the study, results indicate that relative to controls there occurred no leaching of lead from the VSA into the water. In addition, there was no apparent contribution of lead from the VSA with respect to bioaccumulation in the specified tissues of fish (gills, skeleton, skin and scales, muscle, and viscera). There was, however, a significant dose-dependent bioaccumulation of lead in fish housed in aquaria containing a soluble salt of lead (50 and 100 ppb lead as lead nitrate). These studies indicate that bioaccessibility is an important component in determining the ultimate bioavailability of lead.

Keywords: Lead; Bioaccumulation; Vitrification; Sunfish; *Lepomis*

1. Introduction

Recently, several studies have reported the presence of heavy metals in the leachate of incineration ashes or combustion dust. Because of the potential toxicity of these metals, treatment and disposal of the ash has become a national problem. A currently used technology solidifies and stabilizes hazardous waste residues, thus entrapping heavy metals in a glass-like matrix. The waste is prepared through a vitrification process which involves heating the waste to 2500 °F for several hours, thus, reducing it to a glass-like residue containing high concentrations of metals such as lead, cobalt, cadmium, and mercury. This endproduct, a vitrified slagged aggregate (VSA), may have utility as a construction material for use in driveways, parking lots and road beds.

Concern has been expressed that VSA used for such purposes will present a major source of heavy metals contamination to the aquatic environment and to biota living

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in water containing leachate from the VSA. Human exposure to metals by way of ingesting such contaminated biota is also a concern, especially for children, because of their greater sensitivity to lead than adults. Chronic exposure to lead has been shown to cause adverse effects in a variety of organ systems in such ways as interference in heme synthesis, anemia, impaired reproductive function, kidney damage, and delayed neurological physical development. In our laboratory, lead has been selected as an indicator metal in the study of VSA due to recent interest in the effects of exposure to low levels of this metal through drinking water and other means. The connection between exposure to lead and the occurrence of neurobehavioral disorders, particularly in children, is becoming increasingly documented [1]. The medical intervention level for lead in the blood of children was lowered to 20 μg/dl with levels above 10 μg/dl being the recommended cut off for more frequent screening as well as for the initiation of lead poisoning prevention activities in the child's environment [1]. The action level for lead in drinking water has been set at 15 ppb [2].

In a previous study performed in our laboratory [3], VSA containing 3000 ppm of lead was triturated and administered by gavage to mice. Although the acid conditions of the stomach were favorable for the release of lead from the VSA, the blood lead levels of the mice peaked below 14 ppb (8 h after ingestion). Therefore, the lead from the VSA, which reportedly exists as an oxide, was poorly absorbed through the gastrointestinal tract.

The present study again focuses on lead in an effort to examine whether (a) metals from the VSA leach into the aquatic environment and (b) bioaccumulation of lead occurs in sunfish exposed for 100 days to leaching VSA or to low levels of dissolved lead nitrate.

VSA is a black, ceramic material which was obtained as small irregularly shaped fragments which were generally 3–8 mm in diameter. It is devoid of organic content, but contains metals including lead, presumably a type of lead oxide, within a complex structural matrix. Earlier work by this laboratory [3] reported total lead content of the VSA, as determined following strong acid digestion of the pellets, as 2800–3000 ppm lead [4]. It was also demonstrated that only a minimal leaching of lead from the pellets into water occurred, with greatest leaching occurring at pH 3. When VSA was ground into a fine powder, there did not result an increased leachability of lead into water when compared to the original pellet form ($p < 0.05$).

2. Materials and methods

2.1. Aquaria conditions

Ten 60 l aquaria, previously washed with 2% acetic acid and rinsed thoroughly, were each equipped with a pump for the aeration and recirculation of water through glass wool held in a filter box suspended outside the tank. The tanks were filled with deionized water spiked with electrolytes ($NaHCO_3$, 96 mg/l; $CaSO_4 \cdot 2H_2O$, 60 mg/l; $MgSO_4$, 60 mg/l; and KCl, 4 mg/l) to simulate the moderately hard water conditions from which the experimental fish were acquired [5]).

One aquarium was set up with fish and one without fish for each of the 5 experimental conditions. For one such pair of aquaria, 600 g of VSA pellets was placed in a permeable cellulose pouch in each filter box so that the recirculating water percolated through the VSA and returned to the aquarium. A second pair of tanks was similarly set up using 1200 g of VSA for each tank. Four additional tanks contained water spiked with dissolved lead nitrate, two of these with a concentration of 50 ppb lead, and two others with a concentration of 100 ppb lead. These concentrations were chosen because they are in the range of the ambient water criteria for acute exposures to lead set for the protection of aquatic life by the Environmental Protection Agency. These criteria state that the limits of acceptable lead levels for water are 34, 82 and 200 ppb lead when water hardness is 50, 100, and 200 mg/l $CaCO_3$, respectively [6]. The final pair of tanks was used as the control and contained water to which no additional lead was added.

Lead content of the water was monitored daily, and additions of lead to the lead nitrate tanks were made as necessary to keep concentrations at desired levels. In aquaria containing fish, a portion of the water (2/3) was changed twice a week. Water parameters monitored throughout the course of the experiment were as follows: temperature $20 \pm 2\,^{\circ}C$, pH 7.4 ± 0.25, dissolved oxygen 7.96 ± 0.22 mg/l, hardness 74–152 and alkalinity 45–76 mg $CaCO_3$.

2.2. Fish

Sunfish (*Lepomis megalotis*) approximately 4 in in length were used for the study. The fish were collected from a local pond (water lead content 2 ppb) by electroshocking and were allowed to acclimate to laboratory conditions for 3 weeks prior to their introduction into the experimental tanks, each tank containing 12 fish. For the first eight days of the experiment, the fish were fed Purina fish chow. When it was determined that this food had a lead content of 360 ppb lead, cichlid staple diet (Kyorin Co., Ltd, 9 Minami-machi, Himeji, Japan), with a negligible lead content, was substituted. This change in lead content of the food is recognizable in the data.

2.3. Sampling

Fish were removed from the tanks for analysis at the beginning of the study and also were sampled from each experimental tank after 4, 8, 48, 75 and 100 days of exposure. The length of the exposure period conforms to the established 90 day protocol for chronic exposure toxicity testing [7]. Fish were rinsed in deionized water prior to dissection into 5 parts – muscle, viscera, gills, skin with scales, and skeleton (head, spine and fins). The numbers of fish sampled throughout the study were as follows: Initial day of experiment – 3 fish; at 4, 8, and 75 days of exposure – 1 fish from each tank; at 48 days of exposure – 2 fish per tank; at 100 days of exposure – 4 fish per tank. Thus, 5 tissues from each of the 45 fish, or a total of 225 tissue specimens, were analyzed.

Gloves were used during the dissection. The stainless steel knife used for dissection was routinely rinsed with deionized water to maintain the integrity of the sample. The

glassware used was cleaned by soaking in 50% trace metal grade nitric acid overnight, then rinsing with deionized water.

Digestion of the tissues was performed according to the method developed by Schmitt and Finger [8]. Each tissue was placed in capped 50 ml Kimax test tube containing 25 ml of concentrated trace metal grade nitric acid (Fisher) and was heated in a 60 °C waterbath for 40 h. The digest was transferred to a beaker and reduced by evaporation on a hot plate to approximately a 5 ml volume before being brought to a final volume of 50 ml with deionized water. As reported earlier, fat was not completely digested by this method.

Water samples were collected in acid-washed polyethylene test tubes and analyzed immediately.

2.4. Reagents

Lead nitrate, magnesium sulfate, and trace metal nitric acid was obtained from Fisher Scientific (Pittsburgh, PA). Stock solutions of lead for standards and controls were obtained from Fisher Scientific and Ricca Chemical Company (Arlington, TX). Triton X-100 was purchased from Sigma Chemical Co. (St. Louis, MO). Additional salts were obtained from the following suppliers: diammonium hydrogen phosphate, Mallinckrodt, inc. (Paris, KY); potassium chloride, Matheson Coleman and Bell (Norwood, OH); sodium bicarbonate, J.T. Baker Co. (Phillipsburg, NJ), and calcium sulfate, E.M. Science (Gibbstown, NJ).

2.5. Analysis

Analyses were performed on a GBC Model 908 atomic absorption spectrophotometer equipped with a graphite furnace, deuterium lamp background correction, a PAL 3000 autosampler and a computerized data station. Graphite furnace parameters are presented in Table 1. A reduced slit width of 1 nm, was used, and absorbance was measured at 283.3 nm. Pyrolytically coated graphite tubes were used (GBC Scientific, Arlington Heights, IL) for atomization of the samples. For each analysis, 20 µl digest was combined with 10 µl matrix modifier solution (75 g/l

Table 1
Graphite furnace parameters

Step no.	Final temperature (°C)	Ramp time (s)	Hold time (s)	Argon gas flow
1	80	20	20	On
2	150	20	20	On
3	250	15	5	On
4	800	20	20	On
5	800	1	1	Off
6	2400	2	2	Off
7	2500	1	1	On

diammonium hydrogen phosphate, 0.5 ml/l triton-X 100). Aqueous standards and controls in 2% nitric acid solution were prepared from certified lead stock solutions over a range of 1–200 ppb lead.

The analysis of tissue digest spiked with a known concentration of additional lead (60 ppb) demonstrated that virtually all the added lead (96–110%) was detected. Matrix effects on the analyses were thus assessed to be minimal.

3. Results

The concentrations of lead in the water of the various tanks are presented in Table 2. Lead levels in the control tanks and the tanks containing 600 and 1200 g of VSA ranged from 1 to 4 ppb during the course of the experiment.

In spiked aquaria containing lead nitrate without fish, aqueous lead levels were consistently near the target of 50 or 100 ppb throughout the study. However, the comparable aquaria containing fish had broad day to day fluctuations of dissolved lead, sometimes decreasing to 65% of the target lead value, reflecting the removal of lead from the water by uptake into fish, binding to food, and fecal material, etc. Consequently, the experimental design required daily monitoring of lead levels in water and subsequent replenishing of lead through the addition of lead nitrate to the water. Under these conditions, the mean values obtained for the water analyzed for each aquarium containing fish were able to be maintained at the target levels throughout the course of the experiment (Table 2).

The lead content of the fish tissues sampled throughout the experiment are summarized in Table 3. Concentrations are presented as ng of lead per gram of tissue, and the ranges for one standard deviation of the data are presented when 3 or more fish were analyzed. Mean values are presented graphically in Figs. 1 and 2.

The tissue lead content was quite variable from fish to fish even within a single tissue type for individuals simultaneously sampled from a single exposure condition (Table 3). In all experimental groups, there were slight increases in the lead concentrations in all tissues. This increase is attributed to the low but significant lead content in the fish food or aquarium water (3 ppb). However, there was a markedly greater

Table 2
Mean aqueous lead levels for the experimental aquaria ± 1 standard deviation

Exposure Conditions	Lead content (ppb)	
	Aquarium without fish	Aquarium with fish
Control	3 ± 1	3 ± 1
600 g VSA (Low VSA)	3 ± 1	3 ± 1
1200 g VSA (High VSA)	3 ± 1	3 ± 1
50 ppb lead	55 ± 2	48 ± 7
100 ppb lead	106 ± 5	89 ± 14

Table 3
Tissue lead content of sunfish over a 100 day period (ng Pb/g tissue)

Group	Tissue	Day 0[a]	Day 4	Day 8	Day 48[b]	Day 75	Day 100[c]
Control	Gills	490 ± 194		1342	1731	416	2250 ± 1553
	Skeleton	118 ± 62		163	153	83	143 ± 98
	Skin/scales	219 ± 77		587	540	208	556 ± 243
	Muscle	40 ± 14		128	171	77	136 ± 46
	Vicera	72 ± 20		552	581	264	1036 ± 475
Low VSA	Gills	490 ± 194	1386	2920	2078	1434	2708 ± 1083
	Skeleton	118 ± 62	140	176	142	145	167 ± 93
	Skin/scales	219 ± 77	450	952	332	325	485 ± 772
	Muscle	40 ± 14	326	376	120	140	74 ± 51
	Vicera	72 ± 20	669	2333	154	171	940 ± 772
High VSA	Gills	490 ± 194	828	640	1483	1629	2075 ± 1165
	Skeleton	118 ± 62	176	148	167	131	137 ± 101
	Skin/scales	219 ± 77	1045	250	556	389	676 ± 183
	Muscle	40 ± 14	277	100	166	112	108 ± 23
	Vicera	72 ± 20	728	1770	542	379	594 ± 772
50 ppb Pb	Gills	490 ± 194		1880	6327	7012	20641 ± 15318
	Skeleton	118 ± 62		151	1029	1335	1860 ± 1084
	Skin/scales	219 ± 77		656	2191	2361	6847 ± 4876
	Muscle	40 ± 14		207	173	354	330 ± 172
	Vicera	72 ± 20		1047	859	1274	5946 ± 6087
100 ppb Pb	Gills	490 ± 194		3962	6786	11827	28444 ± 16695
	Skeleton	118 ± 62		273	935	–	2134 ± 893
	Skin/scales	219 ± 77		556	1963	3099	7950 ± 5299
	Muscle	40 ± 14		263	120	239	835 ± 399
	Vicera	72 ± 20		21018	470	4281	10058 ± 6979

[a] Mean of 3 fish.
[b] Mean of 2 fish.
[c] Mean of 4 fish.

accumulation of lead in fish exposed to lead nitrate as opposed to fish from the control and VSA groups. This became evident at 48 days into the study for skin with scales, gills and skeleton and by 75 days for viscera and muscle. The lead content of the lead nitrate fish continued to increase until the end of the experiment, whereas lead in the control and VSA fish remained low.

The muscle tissue seemed more responsive than other tissues to the high level of lead in the fish chow used during Day 0–Day 8 of the experiment (Fig. 3). Lead levels of the muscle at Days 4 and 8 were greater in fish from all aquaria than they were in the Day 0 fish. Starting Day 9, low lead cichlid diet was fed to all fish. By Day 48, the muscle lead content had generally decreased from the Day 8 value, and by Day 75, lead content of the Control fish as well as the Low and High VSA fish had continued to decrease, whereas muscle lead content of both 50 and 100 ppb lead nitrate fish increased. By Day 100, muscle lead content of the Control and VSA aquaria remained low, but the muscle lead content of the fish from both lead nitrate aquaria either remained high or continued to increase.

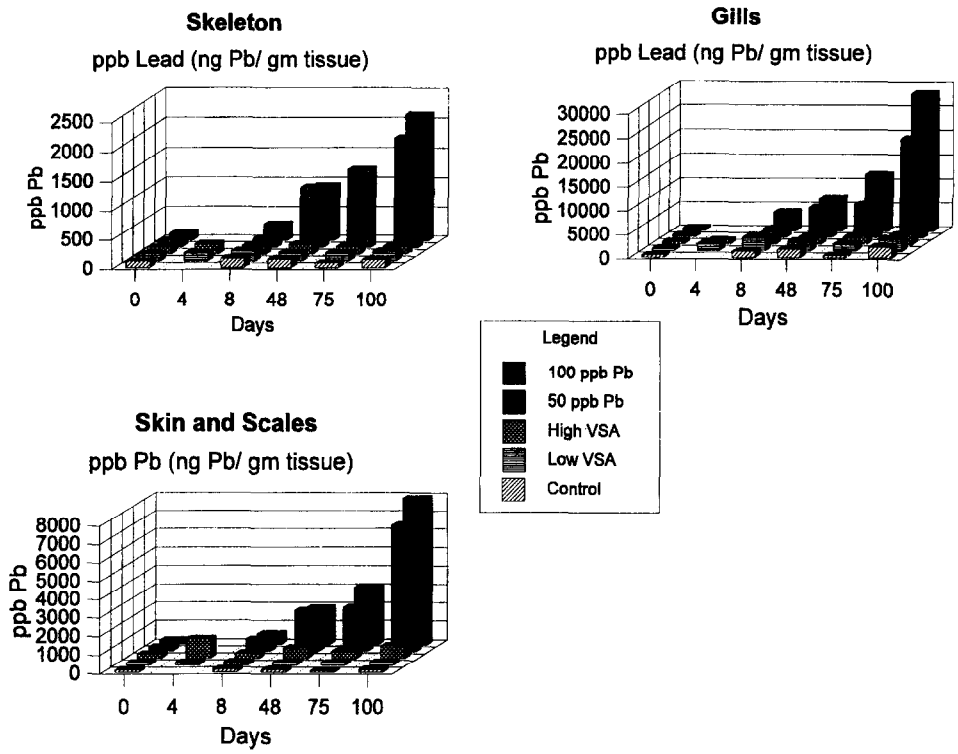

Fig. 1. Lead concentrations of skeleton, gills, and skin and scales in fish exposed to either 600 or 1200 g of VSA for 100 days remained as low as those of the unexposed control fish. However, bioaccumulation of lead is evident in the tissues of fish from the aquaria spiked with 50 or 100 ppb lead as lead nitrate.

The concentration of lead in the 8-day specimen of viscera from the 100 ppb lead aquarium deviated from other values for viscera to a great degree and may perhaps reflect contamination of the sample by incomplete washing of recently ingested food from the stomach prior to processing the visceral tissue.

Lead accumulated differentially in the various tissues with the content generally being lowest in the muscle and the skeleton; intermediate in the viscera and skin with scales, and highest in the gills.

4. Discussion

The data indicate that there is no leaching of lead from VSA under the conditions of this experiment.

A one-way analysis of variance and a Tukey-HSD multiple range test were performed to evaluate the significance at the 95% confidence level of the differences in the mean tissue lead values for the 100 day exposure data. The statistics confirmed that for

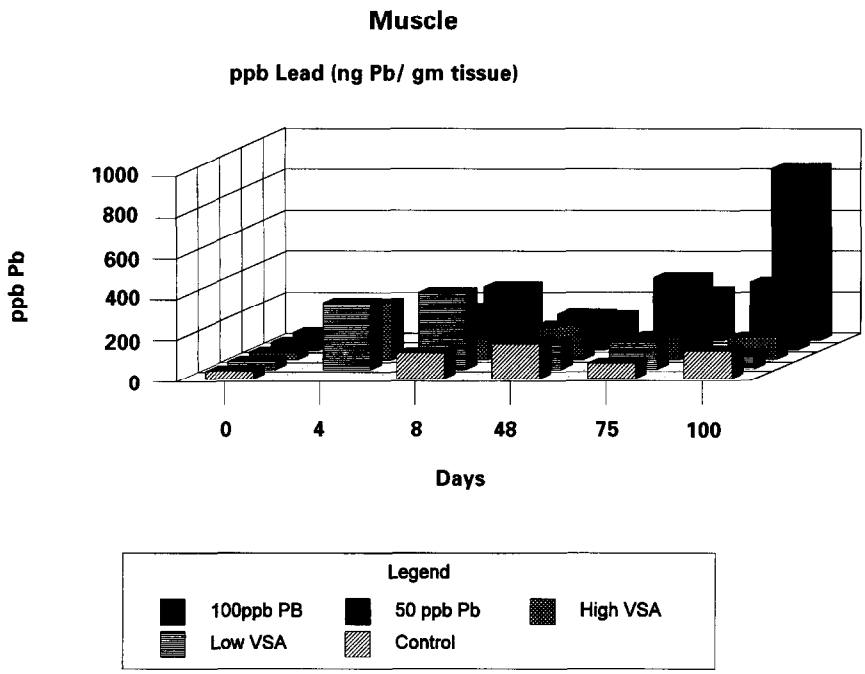

Fig. 2. Lead content of muscle tissues during the course of the study.

each tissue type, the lead concentrations in the fish from the two VSA tanks did not significantly differ from those in the control tank or from each other. Thus, it can be said that no lead originating from VSA was observed to accumulate in any of the tissues of VSA-exposed fish.

Likewise, the aqueous lead levels for aquaria containing 600 and 1200 g VSA remained constant at 1–4 ppb just as did the levels in the untreated control aquaria. Lead did not accumulate in the water, even in the tanks where no fish were present and the water was not changed during the 100 day exposure period.

The considerable effectiveness with which the vitrification process binds the lead is apparent when one considers that the total lead content of VSA which was determined by strong acid digestion, was found to be approximately 4900 ppm lead. Thus, 600 and 1200 g quantities of VSA represent of total addition of approximately 3 and 6 g of lead to the respective experimental aquaria, yet virtually none of this large quantity of lead accumulated in the fish tissue or in the water. The lead, and presumably the other metals captured by molten slag at the end of the waste treatment process, remained incorporated in the cooled aggregate and were highly stable and resistant to leaching.

For the 100-day data from each of the 5 types of tissues, the difference in lead concentration between 100 ppb lead (nitrate) tissue and control tissue was confirmed to be statistically significant ($p < 0.05$). Lead concentrations of the 50 ppb lead (nitrate)-exposed specimens were also significantly higher than those in the controls for the gills, skin with scales, and skeleton, but not for muscle and viscera.

Significant bioaccumulation of lead occurred in fish exposed to waters with both 50 and 100 ppb dissolved lead. These exposure levels are lower by several orders of magnitude than many of the previously reported studies. As a point of reference, 50 ppb lead is the current maximum contaminant level for drinking water set by the EPA [9]. Because fish can so effectively accumulate lead from water with such low dissolved lead content, even minimal contamination with lead can present a significant hazard to organisms when exposure times are extensive.

The tissue lead concentrations from the 50 and 100 ppb lead exposure conditions were not, however, significantly different from each other. Such an observation would result if there was a rate limiting step in the process of accumulation of the lead. There are some indications that this might be the case. For example, Simons [10], by using human red blood cells demonstrated that lead is transported across the cell membrane by the calcium pump. This is an ATP-dependent active transport mechanism following Michaelis–Menten kinetics indicating that lead transport is limited. Only a given quantity of lead can be absorbed per unit time, limiting the rate at which lead is incorporated into the tissues of fish. Because the pump is rate-limited, time of exposure is more meaningful in its effect on bioaccumulation than the actual concentration of exposure. This experiment stimulates the low, chronic lead exposures characteristic of some environmental conditions. This relationship between lead and the calcium pump also leads to the question of whether an increase in dietary calcium would prevent the absorption of lead in humans.

The daily fluctuation of the lead in the aquaria could also be an explanation for the results with the 50 ppb lead not being significantly different from that with 100 ppb lead. The experimental design required aquaria with a lead content of 100 ppb. However, this concentration actually fell as much as 35% during the course of 24 h before it was replenished. Because a constant concentration throughout each 24 h period was not maintained, the actual mean level of lead exposure was somewhat less than the nominal value.

The most recent ambient water criteria for the protection of aquatic life from the toxic effect of lead are hardness-dependent [11]. For freshwater with hardness of 100 mg $CaCO_3$/l, the chronic (4-day average) criterion is 3.2 ppb Pb.

The experiment was performed in water of moderate hardness also simulating the natural conditions of water in the environment from which the fish were collected. This water has a carbonate buffering capacity which can influence the chemical form in which lead is found in water. Dissolved lead, rather than colloidal or certain forms of chemically bound lead, is the form which is toxic to organisms. The buffering capacity of the water must be taken into consideration when evaluating the toxicity of aqueous lead levels. This explains why lead is more toxic in soft water than in hard water.

The tendency for the gills to be a primary site for the accumulation of lead has been reported by others. Liver, kidney and bone have also been reported as sites for the accumulation of lead [12]. This study indicates that skin and scales also accumulate large amounts of lead.

By subtracting the tissue lead levels of the control fish from those of the 50 and 100 ppb lead-exposed fish over the 100 day exposure period, one is able to isolate the net influence of the aqueous lead on the fish tissues. This value, identified as the

Table 4
Accumulated lead and bioconcentration factors for fish exposed to 50 and 100 ppb lead

Tissue	50 ppb lead		100 ppb lead	
	Net lead concentration[a]	Bioconcentration factor[b]	Net lead concentration[c]	Bioconcentration factor[b]
Gills	18391	383	26194	294
Skeleton	1717	36	2045	23
Skin/scales	6291	131	7393	83
Muscle	194	4	699	8
Vicera	4911	102	9022	101

[a] Net lead concentration = lead concentration of lead exposed fish minus lead concentration of control fish.
[b] Bioconcentration factor = concentration of lead in tissue/concentration of lead in water.

accumulated lead in the tissue, is presented in Table 4. Of importance is the fact that edible muscle of the 100 ppb lead-exposed fish has a net accumulated lead value of 699 ppb lead, a level more than twice the 300 ppb level set as the safe level of lead in food by the US Food and Drug Administration [12].

Also of note, is the indication that the edible muscle tissue apparently accumulates (and discharges) lead from food readily. This might be an avenue of future study of particular interest since commercially prepared fish chow used specifically in the rearing of fish for market has been shown to contain significant levels of lead.

Lead concentration in a tissue divided by the mean lead concentration of the water (Table 2) defines the bioconcentration factor for the tissues. The calculated values for this parameter are also presented in Table 4. The great variability of the lead content between individual fish observed in this study is consistent with the findings of other researchers [13, 14] and indicates that generalities about bioconcentration factors must be viewed cautiously. Calculated bioconcentration factors for lead in tissues were, however, found to be lower for muscle, less than a 10-fold concentration, and greatest for gills, approximately a 300-fold concentration.

5. Conclusions

This study examined the possibility of lead leaching from VSA into an aquatic environment and its bioaccumulation in fish exposed to the aggregate for 100 days. The findings are summarized as follows: after 100 days, the lead content in the fish exposed to 600 g of VSA and those exposed to 1200 g VSA were virtually the same as that of the control fish receiving no experimental exposure. Thus, no bioaccumulation of lead from the VSA was observed. Because there occurred no bioaccumulation in the fish tissues or in the water, it can be concluded that there is no leaching of lead from the VSA and the lead apparently remains incorporated in the matrix of the cooled aggregrate where it is highly stable.

Bioaccessibility, which is a measure of the quantity of substance that is free to interact with body tissues, is an important component in the overall determination of the bioavailability of lead. The bioavailability of dissolved lead is normally at 100% indicating that all of the lead would be available for the process of absorption. Prior studies from this laboratory verify that bioaccessibility of lead from VSA under rigorous conditions is only 2–3%. Bioavailability, the percent being absorbed, is a function of bioaccessibility, and uptake is a function of both of these. Because bioaccessibility of lead from the VSA in this study is at a minimum, the percentage of absorption is well below the standard 8% absorption level for adults and the 40% absorption level for children.

Fish exposed to dissolved lead nitrate at concentrations of 50 ppb lead and 100 ppb lead gradually accumulated lead over the entire course of the 100 day exposure period. Final lead content of all tissues in the 100 ppb lead exposed fish was significantly higher ($p < 0.05$) than those of the control and the VSA fish, as were the final lead concentrations of the gills, skin with scales and skeleton of the 50 ppb lead fish. Final lead concentrations in the tissues from the 50 and 100 ppb fish were not significantly different from each other.

The concentrations of lead in terms of ng of Pb per gram of tissue were lowest in muscle and skeleton and were highest in the gills, with skin and viscera being at intermediate levels.

The level of lead attributed to accumulation from the aqueous dissolved lead in the 100 ppb group in the edible muscle of the fish was 699 ppb, more than twice the 300 ppb limit designated by the Food and Drug Administration as the safe level for lead in food. Thus, fish exposed to low levels of lead in their aquatic environment may be unsafe for human consumption.

These data indicate that lead is not released from VSA under the conditions of the study; therefore, VSA may have utility as a construction material in industry. In a previous study, trituration of the VSA resulted in no increase in bioaccessibility of lead regardless of the increased surface area and, thus there was no increase in bioavailability. This, in association with the fact that these small particles of ground VSA also show no leaching, suggests the possibility of using VSA as a road construction material. However, before any further uses can be proposed, a complete study on VSA as a building material must be conducted.

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