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ARAŞTIRMA MAKALESİ **RESEARCH PAPER**

Arsenic Uptake and Depuration by Red Swamp Crayfish, *Procambarus clarkii*

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Atıf yapmak için: **Gedik, K., Kongchum, M. & DeLaune, R.D. (2019).** Kırmızı Bataklık Kerevitinde (*Procambarus clarkii*) Arsenik Alımı ve Depürasyonu .*Anadolu Çev. ve Hay. Dergisi*, ***4***(2)

Abstract: Arsenic (As) is a metalloid and one of the most hazardous elements in the environment. Paddy rice field, which can contain elevated levels of arsenic, also have importance in crayfish production in Louisiana (USA) where crayfish/rice rotation farming is a common application. Such that, in the US, 95% of the total production of crayfish is carried out in Louisiana, and a large portion is consumed there. In this study, arsenic accumulation and depuration in the tissues of crayfish (*Procamborus clarkii*) exposed to different As concentrations were determined. For this purpose, crayfish were exposed to 3 different concentrations of As (0.2, 0,8 and 2 mg As L^{-1}) for 14 days and then kept in As free water for following 14 days. Arsenic concentrations were determined in crayfish tissues (gill, muscle, exoskeleton, hepatopancreas) during both the accumulation (1, 3, 7 and 14th days) and depuration (15, 17, 21, and 28th days) phases. During the accumulation period, arsenic concentration in the tissues was found to increase proportionally with time and exposure concentrations: gill > hepatopancreas > exoskeleton > muscle. During this period, however, the arsenic concentration in the tissues did not reach the equilibrium. In the depuration phase, arsenic elimination varied ranging between 54.63-87.91% in the hepatopancreas, 42.69 to 74.21% in the gills, 35.56-73.55% in the exoskeletons and 26.75-49.84% in the muscles.

Keywords: Accumulation, bioconcentration factor, elimination, kinetic.

Kırmızı Bataklık Kerevitinde (*Procambarus clarkii*) Arsenik Alımı ve Depürasyonu

Öz: Arsenik (As) bir metaloittir ve çevredeki en tehlikeli elementlerden biridir. Yüksek seviyelerde arsenik içerebilen çeltik tarlaları, Louisiana'da (ABD) kerevit/pirinç rotasyonu ile yaygın bir uygulama ile kerevit üretiminde de önemli bir yere sahiptir. Öyle ki, ABD'de toplam kerevit üretiminin %95'i Louisiana'da gerçekleştirilir ve burada büyük bir kısmı tüketilir. Bu çalışmada, farklı As konsantrasyonlarına maruz bırakılan kerevit (*Procamborus clarkii*) dokularında arsenik birikimi ve atılımı belirlenmiştir. Bu amaçla, kerevitler 14 gün boyunca 3 farklı As (0,2; 0,8 ve 2 mg As L^{-1}) konsantrasyonuna maruz bırakılmış ve daha sonra 14 gün boyunca arseniksiz suda tutulmuştur. Arsenik konsantrasyonları kerevit dokularında (solungaç, kas, dış iskelet, hepatopankreas) hem birikim (1, 3, 7 ve 14. günler) hem de depürasyon (15, 17, 21 ve 28. günler) fazlarında belirlendi. Birikim fazında, dokulardaki arsenik konsantrasyonunun zaman ve maruz kalma konsantrasyonları ile orantılı olarak arttığı bulunmuştur: solungaç> hepatopankreas> dış iskelet> kas. Ancak bu dönemde, dokulardaki arsenik konsantrasyonu dengeye ulaşmamıştır. Depürasyon aşamasında arsenik eliminasyonu hepatopankreasta % 54,63-87,91; solungaçlarda % 42,69 – 74,21; dış iskelette % 35,56-73,55 ve kaslarda % 26,75-49,84 arasında değişmiştir.

Anahtar sözcükler: Birikim, biyokonsantrasyon faktörü, eliminasyon, kinetik.

INTRODUCTION

Aquatic ecosystems can be contaminated with agricultural and industrial activities (especially mining) urban wastewater and natural resources. If metal and metalloid levels in the aquatic environments exceed a certain limit value, living organisms in this environment and ultimately human health can be adversely affected (Mandal & Suzuki, 2002; Gedik et al., 2017a; Gedik et al., 2018).

Arsenic (As), a metalloid, is one of the most abundant (20th) and toxic element in nature and it enters the environment in two main ways: natural processes and anthropogenic activities. In general, arsenic concentration is below 10 µg L^{-1} in natural waters, but this value ranges from 0.02-7900 µg L^{-1} in rivers, <0.2-21000 µg L^{-1} in wetlands, ponds and lakes (Mandal & Suzuki, 2002; Azizur Rahman & Hasegawa, 2012). As categorized as a carcinogen for humans (group 1) by International Agency of Research on Cancer (IARC) and has been declared to be largely taken by contaminated drinking water and nutrients (IARC, 2012).

The carcinogenicity of As has therefore led to widespread arsenic detections in rice and rice paddy fields, which are the main nutrients of the world population (Meharg et al., 2009). The paddy fields, one of the largest rice-producing states in the US, contain some As through anthropogenic and natural sources. These paddy fields in Louisiana are also used for the production of crayfish besides rice agriculture (Gedik et al., 2017b). Approximately 95 % (61 000 tons) of total crayfish production in the USA is conducted in Louisiana, of which approximately 70 % is consumed again in this region (McClain et al., 2007; LSAN, 2106). However, human health can be severely affected by excessive consumption of crayfish caught or aquacultured from As contaminated wetlands.

Crayfish have often been used as bioindicators by researchers to determine the bioavailability of metalloids in the aquatic environment, due to its ability to tolerate harsh environmental factors and represent a higher position in the nutrient pyramid for determining biomagnification. Crayfish also have no strict diet choices and can be easily detectable in the aquatic environment. Accumulation of metal(loid)s in crayfish tissues have been studied by numerous research groups (Knowlton et al., 1983; Devesa et al., 2002; Alcorlo et al., 2006; Gedik et al., 2017b). In addition, studies have shown that crayfish rapidly accumulate and depurate Cu, Pb, Zn, and Cd (Naqvi & Howel, 1993; Naqvi et al., 1998; Guner, 2010; Neelam et al., 2010; Soadirini et al., 2012). Current research is mostly based on estimating risks that may affect human health by determining the concentrations of arsenic in foodstuffs. However, there is limited work on As accumulation in crayfish tissues from the water (Naqvi et al., 1990). Therefore, the aim at the present study was to determine the accumulation and depuration of As in the crayfish tissues exposed to different As concentrations.

MATERIAL AND METHODS

Red swamp crayfish (*Procamborus clarkii*) used to determine accumulation and elimination of As in tissues was obtained from a farm representing a monoculture strategy in Crowley, south-central Louisiana, USA (Gedik et al., 2017b). Crayfish were 4.30±1.58 g mean weights and 5.96±0.78 cm mean lengths. All crayfish were acclimated to the laboratory environment in the aquariums filled with tap water at 21±1 ºC for daily with 12 hours' light/dark period for a total of 12 days. Thereafter, crayfish were placed in the aquariums containing different concentrations arsenic solutions prepared using KH_2AsO_4 (0.2, 0.8 and 2 mg As L^{-1}; Figure 1). Temperature and pH of water were measured during the experiment using pH meter (Accuamet AP62).

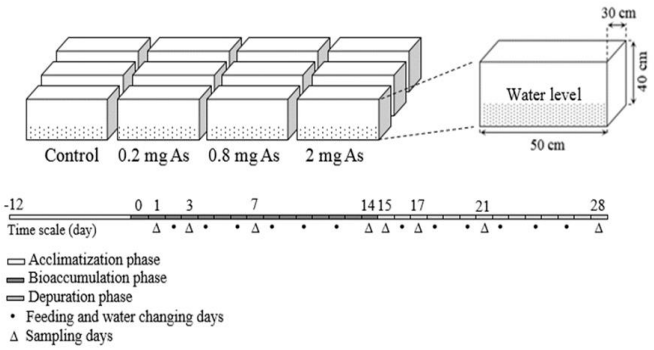

Figure 1. Experimental setup and sampling diagram.

Thirty crayfish were placed in each aquarium and tap water was used as a control group. Three crayfish were taken from each aquarium on days 1, 3, 7 and 14. After a certain period of feeding, solutions at the same concentrations were prepared and replaced every two days to be able to eliminate the changes in the concentrations and pollution of the solution (Figure 1). To check the As concentrations of solutions, water was also sampled after preparing a fresh solution. At the end of the bioaccumulation phase (day 14), the solutions were removed from the aquariums and replaced with tap water (5 l) go for the elimination phase. During the elimination phase, the same procedure (crayfish and water sampling, feeding and water changing) were carried out (Figure 1). Water samples were filtered through 0.45 µm pore size (Ø) filters and stored at 4 ºC until analysis for arsenic (EPA, 1994). The tissues (exoskeleton, hepatopancreas, gill, and muscle) were dissected from sampled crayfish and dried in the oven (< 40 °C) for constant weight. Then, the dried samples were homogenized. A part of homogenized samples was placed into Teflon digestion vessels (MarsXpress, CEM). After that concentrated nitric acid (Suprapur 65 % EMD Millipore #100441) were added depending on digested samples weight and then the samples were digested for 30 minutes using microwave digestion unit (MARS 5, CEM) at 200 °C (Gedik et al., 2017b). After digestion, samples were allowed to cool in the fume hood and transferred to tubes. The samples were diluted with ultrapure water and then kept at 4 ºC until

analysis. Oyster tissue (1566b) from the National Institute of Standards and Technology was used as standard reference material for controlling the digestion results. Recoveries ranges for arsenic from SRM were calculated as 92–96 %. Arsenic concentrations in the samples were detected by ICP-OES (Spectro Arcos, Germany). The Blanks and certified samples were run during each analysis for checking quality control of ICP-OES measurements. All experiment was performed three times. Digested tissues values were calculated based on a dry weight basis (mg kg $^{-1}$ DW).

Data Analysis

Firstly, data were checked using normality and equal variance test. Secondly, two-way ANOVA was used to determine the effect of solutions have a different arsenic level and exposure time on the arsenic level in different tissues. These tests were performed using SigmaPlot 13 software (Chicago, USA).

The uptake and elimination of arsenic in red swamp crayfish tissues were calculated using first-order kinetics model decelerated by OECD Test Guideline 305 (2012). Calculation of elimination and uptake rates values were given as follows equation:

$$C_{cf} = C_w \text{ x } \frac{k_1}{k_2} \text{ x } \left(1 - e^{-k_2 t}\right) \text{ when } 0 < t < t_c \cdot (1)$$

Where C_{cf}: concentration in crayfish at time t (mg kg $^{-1}$),
C_w = concentration in water during the uptake phase (mg L^{-1}),
k_1 = uptake rate (l kg $^{-1}$ day $^{-1}$),
k_2 = depuration rate (day $^{-1}$),
t = time (days),
t_c = time at the end of the uptake phase (days).

RESULTS AND DISCUSSION

Water temperature and pH values ranged between 20.8-21.2 °C and 7.16-7.21, respectively during the study. During the accumulation and elimination of As in crayfish tissues, these changes were not found to be statistically significant. The mean weight of crayfish used was 4.30±1.58 g before exposure experiment, increasing to the 4.45±1.03 g (n=27) after 28 days. Similar weight increase trend was detected in the control group as the mean weight of crayfish used in this group (tap water conditions) was 4.40±1.86 g and increased to 4.94±1.54 g after the same period of time (n=12). Although there was detected to be a weight increase in crayfish samples after 28 days, it was found that there was no statistically significant difference between the groups.

When the samples taken from different concentrations of arsenic solutions (0.2, 0.8, and 2 mg L^{-1}) were tested, arsenic levels were found to be 0.19±0.01, 0.78±0.02 and 1.98±0.04 mg L^{-1}, respectively. In the control group, the amount of arsenic was below the detection limit (< 2.0 µg L^{-1}). Changes in arsenic concentrations in the crayfish tissues sampled from the control group during the experimental period were not statistically significant different ($p>0.05$).

The changes in the arsenic levels during bioaccumulation and elimination phases in the tissues of crayfish sampled on days 1, 3, 7, 14, 15, 17, 21 and 28, which are exposed to different arsenic concentrations, are given in Figure 2. Arsenic accumulation in all the tissues increased during the bioaccumulation phase (0-14 days). Arsenic accumulation reached the highest level on the day 14 and decreased during the elimination phase (15-28 days) (Figure 2). The As values were calculated to be 4.70 mg kg $^{-1}$, 11.39 mg kg $^{-1}$, and 18.24 mg kg $^{-1}$ in the gill tissues, 0.76 mg kg $^{-1}$, 1.61 mg kg $^{-1}$ and 2.76 mg kg^{-1} in the muscle tissues, 3.27 mg kg $^{-1}$, 6.40 mg kg $^{-1}$ and 8.71 mg kg^{-1} in the hepatopancreas and finally 1.43 mg kg $^{-1}$, 1.43 mg kg $^{-1}$ and 2.80 mg kg^{-1} in the skeleton of crayfish exposed to 0.2, 0.8, 2 mg L^{-1} arsenic concentrations, respectively. In the present study, the temporal variations of the arsenic values determined in the crayfish samples taken at days 1, 3, 7 and 14 exposed to different arsenic concentrations through 14 days were significant ($p < 0.05$). Neelam et al., (2010) determined that the optimum accumulation level for Cu and Zn which are among the essential metals is 32-52 ppm, but for the non-essential element Pb, the accumulation rate increased proportionally to the solution concentration. Knowlton et al., (1983), Naqvi et al., (1990) and Guner, (2010) have also determined that the metal levels in the solution are important for the accumulation of metals which is not essential for the crayfish metabolism. In this study the highest arsenic value was measured in samples exposed to 2 mg L^{-1} arsenic solution at the end of 14 days. Anderson et al., (1997) and Naqvi et al., (1990) was reported that the maximum accumulation was found to be in the gills of crayfish exposed to metal and metalloid solutions. In this study, the level of arsenic accumulated in the tissues of crayfish exposed to solutions which have different arsenic concentrations was in the order of G > H > E > M. The temporal variation of measured As values were significant ($p<0.05$) in samples taken on days 1, 3, 7 and 14 from crayfish kept in tap water to determine arsenic depuration. At the end of the elimination period (14 days), it was detected that the arsenic levels in the crayfish tissues were changed with different arsenic concentrations besides different tissues. Arsenic depuration was found to vary from 42.69 to 74.21 % in the gills, 26.75-49.84 % in the muscles, 54.63-87.91 % in the hepatopancreas and finally 35.56-73.55 % in the exoskeletons. Data obtained in our study was in parallel with the results for the arsenic elimination of studies regarding arsenic, cadmium and lead elimination in crayfish (Naqvi et al., 1990; Naqvi et al., 1993; Guner, 2010; Soedarini et al., 2012).

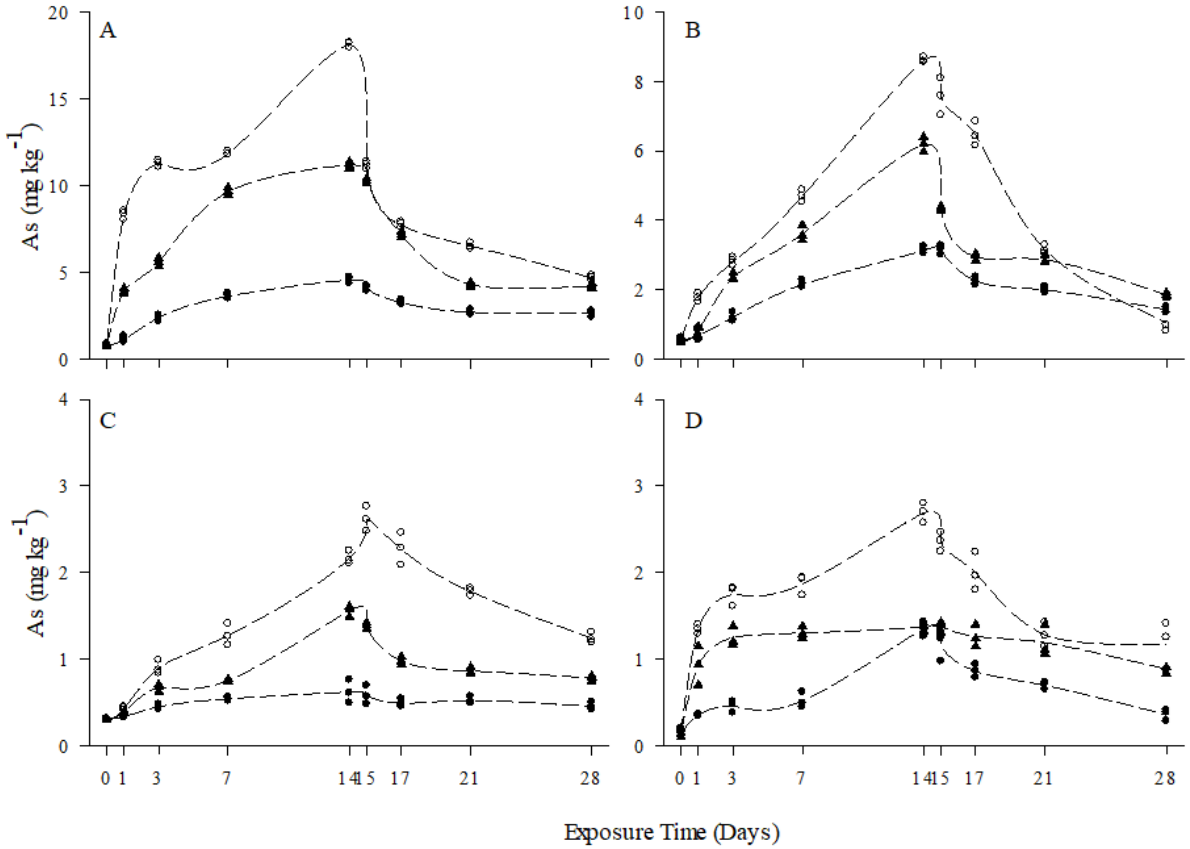

Figure 2. Accumulation and elimination of arsenic in crayfish tissues. Each data point represents a measurement of an individual crayfish (dry weight). A: gill, B: hepatopancreas, C: muscle, D: exoskeleton. Symbols (●, ▲, ○) indicate the different crayfish groups exposed to 0.2, 0.8, 2 mg As L^{-1}, respectively. The crayfish were exposed to the As solutions from days 0 to 14 (accumulation), and transferred to the tap water days starting on the 14th to 28th (elimination)**, (n=3).**

The first order kinetic model was used to calculate the uptake and elimination of arsenic in red swamp crayfish tissues. Firstly, elimination rate (k_2) values were obtained using plot of ln (C_{cf}) against time for the elimination phase and then checked r^2 values confirmed (OECD, 2012) that the depuration follows first-order kinetics in this case except for the r^2 value for the 0.2 mg L^{-1} muscle tissue is low (Table 1). Then, uptake rate values were calculated for each time (1, 3, 7, and 14 days) using Equation 1. These values are given in Table 1. Data show that k_1 values were generally fluctuating with increasing exposure time except for gill and exoskeleton tissues which are directly contacted with water. Bioconcentration factors (BCF) also were estimated as the ratio of the As uptake (k_1) and elimination rates (k_2) for crayfish tissues exposed to As at different concentrations (Table 1). The maximum BCF value (74.47 l kg^{-1}) was calculated in gill tissue, while the minimum was in muscle tissue (1.88 l kg^{-1}). In addition, the growth dilution was also calculated to eliminate the errors for BCF that might be caused by the increase in crayfish weights during the experiment. Therefore, growth dilutions were obtained using the plot of ln (1 / Crayfish weight (kg)) against time. Growth dilution rates were calculated as 0.0079, 0.0037, and 0.0011 for 0.2, 0.8, 2 mg L^{-1} exposure groups, respectively. Then, growth corrected BCF were calculated and given in Table 1. It was determined that the effect of growth dilution rates on BCF was varied between 0.62 % and 43.9 %. Since the fluctuations or apparent decline in k_1 values were detected as the time progresses in the uptake phase, the data did not fit to first order kinetics model. Calculated BCF and k1 values for different crayfish tissues were in the order of gill > hepatopancreas > exoskeleton > muscle (Table 1). Anderson et al., (1997) reported that the gills in crayfish are in direct interaction with the water to support ion and gas exchange. That might be the reason for the arsenic levels in the gills of crayfish exposed to different arsenic concentrations were found higher than that of other organs. In addition, different physiological processes have occurred in hepatopancreas and it contains metal-binding proteins, hence metal(loid)s are stored in this tissue. (Alcorlo et al., 2006; Soedarini et al., 2012; Gedik et al., 2017b). Bioconcentration factor is generally used by researchers in bioavailability studies conducted under controlled conditions (Weisbrod et al., 2007; Soedarini et al., 2012). The decrease in the BCF values in the crayfish was found to be proportional to the increase in the arsenic concentration in the exposed solutions based on the BCF values calculated for As in the crayfish tissues. Soedarini et al., (2012) has been reported that the BCF value is inversely correlated to the crayfish body size and the metal concentration in the exposed solution.

Table 1. Arsenic uptake (k_1), elimination (k_2) rates and bioconcentration factor (BCF) in different tissues of the crayfish (*Procamborus clarkii*) calculated using first-order kinetics model

Time (Days)	Calculated k_1 (L kg^{-1} day^{-1})			k_2 (day^{-1})			Kinetic BCF (L kg^{-1})			Kinetic BCF growth corrected (L kg^{-1})		
	L	M	H	L	M	H	L	M	H	L	M	H
G				0.038	0.073	0.082						
1	1.80	4.06	3.19	(0.75)	(0.80)	(0.80)	47.37	55.62	38.90	59.80	58.33	39.43
3	2.83	2.23	1.97				74.47	30.55	24.02	94.02	32.04	24.35
7	2.31	2.02	1.04				60.79	27.67	12.68	76.74	29.02	12.86
14	1.74	1.48	1.04				45.79	20.27	12.68	57.81	21.26	12.86
M				0.018	0.046	0.048						
1	0.17	0.11	0.06	(0.33)	(0.75)	(0.88)	9.44	2.39	1.25	16.83	2.58	1.28
3	0.25	0.16	0.11				13.89	3.48	2.29	24.75	3.76	2.35
7	0.18	0.09	0.08				10.00	1.96	1.67	17.82	2.11	1.71
14	0.13	0.15	0.09				7.22	3.26	1.88	12.87	3.52	1.92
H				0.057	0.073	0.155						
1	0.83	0.37	0.66	(0.92)	(0.83)	(0.98)	14.56	5.07	4.26	16.90	5.32	4.29
3	1.20	0.85	0.47				21.05	11.64	3.03	24.44	12.21	3.05
7	1.40	0.70	0.49				24.56	9.59	3.16	28.51	10.06	3.18
14	1.34	0.81	0.71				23.51	11.10	4.58	27.29	11.64	4.61
E				0.092	0.032	0.061						
1	1.06	0.99	0.62	(0.94)	(0.82)	(0.79)	11.52	30.94	10.16	12.60	34.62	10.35
3	0.59	0.48	0.29				6.41	15.00	4.75	7.02	16.78	4.84
7	0.36	0.23	0.15				3.91	7.19	2.46	4.28	8.04	2.50
14	0.76	0.14	0.14				8.26	4.38	2.30	9.04	4.90	2.34

G: gill, M: muscle, H: hepatopancreas, E: exoskeleton. L: 0.2 mg L^{-1}, M: 0.8 mg L^{-1}, H: 2 mg L^{-1} As exposure concentration, BCF= (k_1/k_2), R^2 values is given in parentheses.

Results of two-way analysis of variance examined time of exposure and concentrations of the arsenic accumulation in the different tissues of crayfish. According to the analysis, it was determined that the effect of time and concentration differences on the arsenic accumulation in tissues, not only time and different concentration but also interaction between time and arsenic exposure, was significant for crayfish tissues (Table 2).

Table 2. Results of two-way ANOVA for the effect of time and arsenic exposure concentration on the arsenic accumulation levels in different tissues of crayfish (*Procambarus clarkii*).

Dependent variable	As exposure	Time	As*Time	R^2
As in gill	p<0.05	p<0.05	p<0.01	0.99
As in muscle	p<0.01	p<0.05	p<0.05	0.98
As in hepatopancreas	p<0.05	p<0.01	p<0.01	0.99
As in exoskeleton	p<0.01	p<0.01	p<0.01	0.98

CONCLUSION

The results of this study showed crayfish maintained in different concentrations of arsenic solutions. The highest arsenic level in crayfish tissue was measured at the end of the longest exposure period (14th day) for crayfish kept in the solution with the highest amount of arsenic (2 mg L^{-1}). Depuration of arsenic from crayfish tissues was found to vary from 26.75 % to 87.91 %.

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