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Research Article

The Role of Zeolite in Alleviating Lead Toxicity in *Tubifex tubifex*

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ABSTRACT
Low-cost, environmentally friendly adsorbents and catalysts have gained importance in research due to their impact on heavy metal in recent years. This study aimed to investigate the ameliorative effect of the adsorbent properties of zeolite on lead bioaccumulation in Tubifex tubifex (Müller 1774). The oligochaete worms were exposed to 0.1 µg/l Pb, 0.1 µg/l Pb + 0.1 µg/l zeolite, and 0.1 µg/l Pb + 1 µg/l zeolite mixtures for 24, 48, and 96 hours. Lead accumulation in the whole body and environmental media of T. tubifex was determined using inductively coupled plasma mass spectrometry (ICP-MS). Lead toxicity increased with longer exposure durations. In all groups, lead accumulation was statistically significant at all exposure times. The presence and concentration of zeolite significantly reduced lead content in T. tubifex.

Keywords: *Bioaccumulation; ICP-MS; Lead; Oligochaete worm; Zeolite.*

Introduction

Lead is one of the major pollutants, along with thirteen other metals and semi-metals, and is not necessary for the metabolic needs of organisms [1]. It is released into the environment by industrial facilities producing lead-acid batteries, lead paint, lead plumbing, lead printing materials, and through lead mining [2], [3]. Lead can be extremely harmful to ecosystems as it is neither metabolized nor biodegradable [4]. Even at very low concentrations, non-essential lead can affect the nervous system and pose public health risks due to its neurotoxicity [5]. Furthermore, elevated lead levels in the environment have been linked to issues such as hearing impairments, behavioural abnormalities, and neuromuscular weakness, all of which severely impact both humans and wildlife [6].

Many studies have shown that heavy metals adversely affect species in aquatic ecosystems, including both invertebrate [7] and vertebrate [8], as well as plants and animals that make up the habitat components over time [9]. *T. tubifex* plays a significant role in aquatic ecosystems, due to its diverse fatty acids, essential amino acids, carotenoid pigments, and nutritional value as a protein source [10]. While widespread ecophysiological effects of lead have been observed to cause changes in the body parts of *T. tubifex* [11], changes in survival rates have been noted in acute toxic situations [12].

In addition to understanding lead's harmful effects, studies focused on monitoring lead levels using various environmental techniques and reducing its concentration in aquatic environments have become crucial [13], [14]. Various low-cost, environmentally friendly adsorbents have been employed to remove heavy metals from aquatic ecosystems and mitigate their harmful effects [15], [16]. Among these, clinoptilolite, a type of zeolite, has proven effective in removing lead, cadmium, copper, cobalt, chromium, zinc, nickel, and mercury [13], [17], [18]. This study aimed to investigate the effects of different concentrations of zeolite on reducing lead toxicity in *T. tubifex* and its bioaccumulation over 24, 48, and 96 hours.

Materials and Methods

Experimental Animals

In bioconcentration studies, the oligochaete worm *Tubifex tubifex* has become a prominent model species due to its wide distribution, ease of experimental setup, and high reproduction rate [19], [20]. Typically, *T. tubifex* is found in water bodies with low dissolved oxygen, high organic matter, turbidity, and muddy waters [21]. Numerous physiological parameters have demonstrated its high sensitivity to environmental stress [21], [22]. For these reasons, *T. tubifex* was chosen as the experimental animal [22].

Samples were obtained from a specialized fish breeding centre and acclimatized in a plastic tub containing water and sediment for two weeks. During the adaptation period, stock samples were maintained in dechlorinated tap water, replenished regularly and kept at 18 °C. The stock tanks were fed spirulina aquatic food disks once daily under a 12-hour light and 12-hour dark photoperiod [19].

Table 1. The validation parameters of analytic techniques of lead

Wave Lengths	Recovery (%)	Detection limit (µg/l)	Quantification limit (µg/l)	Relative standard deviation (%)	R^2
219.885	98.08	2.9	1.5	1.9	0.97

Experimental Design and Chemicals

Since there were four experimental groups to be examined in total, four experimental tanks were used. The static experimental application was maintained to collect and separate oligochaete worms in all experiment durations. Experiments were carried out with a total of 240 oligochaete worms, 60 for each group. At the beginning of the experiment, the individual mean and standard error of body length and weight were determined as 1.20 ± 0.01 mm and 0.84 ± 0.01 mg. The investigations were carried out using 1000 mL of dechlorinated water in clear acrylic containers. By examining the permitted limit values of specific contaminants for inland surface water sources, the applied lead limit was taken into consideration [3]. The 0.1 µg/l concentration of lead (T1) was injected into the first experimental tank. A mixture of 0.1 µg/l Pb + 0.1 µg/l zeolite and 0.1 µg/l Pb+1 µg/l zeolite were injected into the second and third groups, respectively. No chemicals were injected into the final experimental tank, which served as the control group. During the experiment, the worms were not fed to prevent the interaction of lead with the organic food. Zeolite (25 µm, min. purity: 85 %, cation exchange capacity: 170 meq/100 g, Rota Mining Co., Türkiye) was used to reduce the toxic effect of lead nitrate (II) ($Pb(NO_3)_2$, purity: 99 %, Sigma-Aldrich Co., Germany) on oligochaete worm. For lead and zeolite mixtures, 20 samples were taken from each aquarium, considering the test periods of 24, 48 and 96 hours, and kept in an oven at 150 °C for 48 hours until they reached constant weight (Sartorius CP-224S). The worm samples, whose weights were determined, were transferred to test tubes; a mixture of nitric acid (Merck, % 65, S.G: 1.40) and perchloric acid (Merck, % 60, S.G: 1.53) (2v/v) was added and burned at 105 °C for 3 hours [23]. Experimental media samples were taken daily into 0.45-µm cellulose acetate syringe tubes and acidified with 1% nitric acid to analyze lead levels. Inductively coupled plasma mass spectrometry (Agilent 7500ce, Octopole Reaction Systems, Agilent Technologies, Japan) was used to measure lead concentrations in the body and tank mediums of oligochaete worm. The operational features of the device for lead determination are given in Table 1. By introducing the chemical from the stock solution to the test environment at concentrations established by standard procedures, the lead stock solution was prepared daily [24]. The physical and chemical properties of the experimental tanks are shown in Table 2.

Table 2. Water quality characteristics of experimental medium

Parameters	Units	Values
DO	mg/l	7.44
pH	-	7.09
Conductivity (EC)	µS/cm	214.2
Total organic carbon (TOC)	µg/l	0.81
Sulfate (SO_4^+)	µg/l	5.10
Ammonium (NH^{4+})	µg/l	0.01
Total hardness ($CaCO_3$)	mg/l	275

Statistical Analysis

Lead accumulation calculations and graphs of analytical measurement values obtained from experimental analyses were made in Microsoft Excel software. All the statistical analyses were performed using SNK and variance analyses. The data was collected using SPSS statistical software (V 27.0.1.0, IBM, Corp., USA) for SNK (Student Newman Keul's Test) and variance analysis applications [25]. Letters a, b, c, d, and x, y, z show differences among treatments and exposure durations, respectively. Data shown with different letters are significant at the *$P \leq 0.05$ level* [26].

Table 3. Statistical evaluation of lead accumulation in *T. tubifex*

Treatments	Exposure time (hours)		
	24	48	96
Control	ax ↔	ay ↔	ay ↔
T1	bx ↑	by ↑	bz ↑
T2	cx ↓	cy ↓	cz ↓
T3	dx ↓	dy ↓	dz ↓

Notes: *(↑: increase, ↓: decrease, ↔: no change).*

Results and Discussion

To determine the effect of zeolite on lead accumulation in oligochaete worms, SNK and variance analysis were performed on the data, and the results are given in Table 3. Statistical differences were determined at the level of ≤0.05 between the data indicated with different letters in the table. It was determined that the accumulation of lead concentration in the medium increased in *T. tubifex* at all tested times (24, 48, and 96 hours). Lead accumulation showed a time-dependent decrease with increasing zeolite concentration applied (P≤0.05). Lead accumulation in *T. tubifex* in the treatment tanks was calculated as a percentage change compared to the control (Figure 1). The remediation effect of zeolite at 0.1 µg/l (T2) and 1 µg/l (T3) on lead levels in *T. tubifex* showed a decrease of 25.7 % (T2) and 54.3 % (T3) in 24 hours, 16.9 % and 56.9 % in 48 hours, and 30.1 % and 62.3 % in 96 hours compared to (T1).

The most significant harmful effects of lead on aquatic organisms are ion imbalance, hypoxia brought on by a blockage of oxygen uptake in the gill lamellae, and inhibition of the activity of several enzymes essential to metabolic processes [27]. It has been reported that the lead level in *Heteropneustes fossilis* and *Cyprinus carpio* exposed to the effect of lead and zeolite mixtures decreased [28], [29]. In many studies (Table 4), it was observed that the lead and other toxicant residues in the organism and ambient decreased in the presence of zeolite [30]. As a result, various chelators have been used in many studies to prevent lead accumulation (Table 5). In this study, the cation exchange capacity of zeolite provided an economical solution to prevent the toxic effects of lead accumulation in *T. tubifex* and to ensure the metabolic regulation of the organisms. This situation is similar to the decrease in lead accumulation in *T. tubifex* in the presence of zeolite.

Table 4. Protective effects of zeolite on aquatic animals against toxicity of chemicals

Organism	Habitat	Chemicals	Remediation	Ref
Heteropneustes fossilis (Bloch, 1794)	When enough rainwater accumulates, this species can breed in ditches, ponds, and abandoned ponds, but it prefers confined waters during the monsoon season.	Lead(II) nitrate, [$Pb(NO_3)_2$]	Zeolite effect reduced liver soluble protein, RNA, glycogen contents, cholesterol levels, and negative effects on body weight.	[28]
Oreochromis mossambicus (Peters, 1852)	Habitats of this species include slow-flowing water bodies such as lagoons, rivers, dams, the upper reaches of estuaries, and coastal lagoons.	Cupric sulfate, [$CuSO_4$]	Fish RNA:DNA ratios improved as a result of zeolite treatment.	[31]
Cyprinus carpio (Linnaeus, 1758)	This species is widespread in eutrophic waters of Eurasian lakes and large rivers.	Ammonia, [NH_3]	The application of natural zeolite prevented fish deaths.	[32]
Catla catla (Hamilton, 1822) *Labeo rohita (Hamilton, 1822* *Cirrhinus mrigala (Hamilton, 1822*	The habitats of this species are freshwater rivers and lakes in Northern India, Bangladesh, Myanmar, Nepal and Pakistan.	Ammonia, [NH_3]	Zeolite controlled toxicity caused by ammonia concentration in fish transport containers, maintained pH balance, and increased oxygen supply.	[33]
Chironomus riparius (Meigen, 1804)	These chironomid flies are naturally occurring and the most prevalent organisms in aquatic environments.	Thiacloprid, [$C_{10}H_9ClN_4S$]	Zeolite reduced the acute toxicity of thiacloprid	[34]
Oncorhynchus mykiss (Walbaum 1792)	These species are native to the North American continent and prefer clear, fast-flowing streams and rivers.	Iron(II) chloride, [$FeCl_2$]	Oxidative stress levels of fish under the influence of zeolite decreased.	[35]

It is known from geological research that zeolite minerals form as a result of chemical reactions between volcanic material accumulated in terrestrial environments, fresh-salt wetlands, open or closed lake systems, bitter water lakes and thermal water sources, and coastal-deep sea systems in these regions with ambient water [36]. This also provides information into how volcanic formations offer coacervate benefits to the biodiversity in aquatic ecosystems [37].

Common uses of zeolite include the treatment of drinking water waste, removal of toxic substances from the environment in fish farms, reduction of toxic gas emissions, use as supplementary feed in terrestrial animal husbandry, applications in the cosmetics industry, and many other areas [38 – 40]. In recent years, grid-based methods such as the probe molecule method and scanning electron microscopy with energy-dispersive X-ray spectroscopy have been employed to understand the scalability of zeolite composition and particle size morphology (shape). Zeolite, as a sorbent of natural origin, is readily available and already present in various habitats with organisms. Despite no negative ecological effects being observed, studying its sensitive effects on biota remains important [41 – 43].

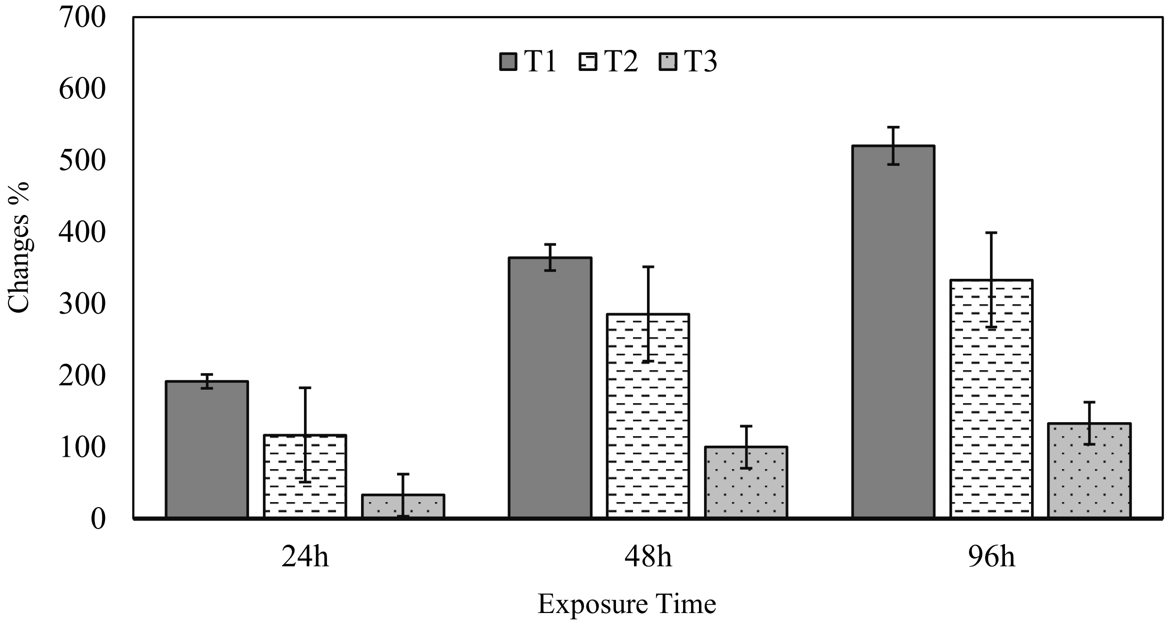

Figure 1. Change of lead accumulation in *T. tubifex* concerning control (%).

Table 5. Comparison of the retention capacity of zeolite with other adsorbents

Adsorbents	Adsorbent origin*	Area of use	Result	Ref
Calcite Zeolites	Soil and ore materials	Heavy metal removal	Maximum desorption of various metals was achieved by zeolite	[44]
Clays Zeolites	Soil and ore materials	In radioactive waste management	The fastest rate of radioactive absorption has been obtained in the zeolite applications.	[45]
Fly ash Zeolites	Industrial waste Soil and ore materials	Heavy metal retention	Heavy metals were retained in zeolite with a continuously increasing trend.	[46]
Chitosan Zeolites	Sea materials Soil and ore materials	Reduction of heavy metals and pathogens	Zeolite was found to have higher reduced metal bioavailability than chitosan.	[47]
Clove oil Zeolites	Agricultural products Soil and ore materials	Transport of aquatic products	Zeolite reduced NH_3 levels	[48]

Notes: Classification of absorbents origins [49].*

Conclusions

The results indicated that zeolite protects *T. tubifex* against lead toxicity. The lead levels in *T. tubifex* significantly increased when exposed to lead alone, whereas lead accumulation decreased when exposed to a lead + zeolite mixture, depending on exposure duration and the environmental concentration of zeolite. This reduction is likely due to zeolite's ion exchange properties, which lower lead concentrations in the environment and subsequently in the organism. In this context, zeolites are considered harmless for *T. tubifex*, an important food source for fish, and they may help reduce the risk of lead contamination transmitted through the food chain.

Conflict of Interest

The author declares no conflict of interest.

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