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Effects of Phosalone on Mineral Contents and Spinal Deformities in Common Carp (*Cyprinus carpio*, L.1758)

Ekrem Sanver Çelik[1,*], Hasan Kaya[1], Sevdan Yılmaz[1]

[1] Çanakkale Onsekiz Mart University, Fisheries Faculty, 17100, Çanakkale, Turkey.

* Corresponding Author: Tel.: +90.286 2180018-1559; Fax: +90.286 2180543;
E-mail: sanver_celik@hotmail.com

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Abstract

The objective of this study was to evaluate the effect of phosalone on some mineral contents, morphological abnormalities and spinal deformities in common carp (*Cyprinus carpio*, L.1758). Fish were exposed to 0.15, 0.30 and 0.60 mg L^{-1} of phosalone using a semi-static exposure regime for 14 days. Experiments were conducted in triplicate for control treatments and phosalone concentrations. At the end of the experiment, abnormal swimming and lethargy was observed in fish exposed to 0.30 and 0.60 mg L^{-1} phosalone concentrations. The percentage morphological abnormalities of the control and phosalone treatment groups (0, 0.15, 0.30 and 0.60 mg L^{-1}) were 0%, 0%, 35.33% and 65.00%, respectively. In direct radiography, a decrease in kyphosis at the thoral spine and a lordotic anomaly at the caudal spine were detected in fish exposed to the 0.30 mg L^{-1}. In addition, deformity at the thoraco-abdominal junction of the spine and decreased lordotic curvature in the caudal region in the treatment group of 0.60 mg L^{-1} were observed. Calcium and potassium levels in the spinal tissue of fish significantly decreased in the 0.30 and 0.60 mg L^{-1} phosalone concentrations. While sodium concentration in the spinal tissue significantly decreased in all the phosalone-treatment groups, magnesium was significantly reduced only in the 0.60 mg L^{-1} phosalone concentration group. Phosalone pollution may cause various ill effects on aquatic ecosystem, including spinal deformities. Therefore, it has to be kept under acceptable concentrations in aquatic environment.

Keywords: *Cyprinus carpio*, phosalone, toxicity, radiography, mineral analysis.

Sazan Balığı (*Cyprinus carpio*, L.1758)'nda Spinal Anormallikler ve Mineral İçeriği Üzerine Phosalone'un Etkisi

Özet

Bu çalışmada, sazan (*Cyprinus carpio*, L.1758) balığının bazı mineral içerikleri, morfolojik anormallikleri ve omurga deformasyonu üzerine phosalone'nin etkisi araştırılmıştır. Balıklar 0,15, 0,30 ve 0,60 mg L^{-1} phosalone konsantrasyonlarına semi-statik olarak 14 gün boyunca maruz bırakılmıştır. Çalışma kontrol grubu ve phosalone konsantrasyonları için üç tekerürlü olarak yürütülmüştür. Deneme sonunda 0,30 ve 0,60 mg L^{-1} phosalone konsantrasyonlarına maruz bırakılan balıklarda anormal yüzme ve letarji görülmüştür. Morfolojik anormallik yüzdesi, kontrol ve phosalone maruz bırakılan gruplarda (0, 0,15, 0,30 ve 0,60 mg L^{-1}) sırasıyla %0, %0, %35,33 ve %65,00 olarak bulunmuştur. Direk radyografiye bakıldığında 0,30 mg L^{-1} konsantrasyona maruz bırakılan balıklarda thoral omurga kifozunda azalma ve kaudal omurgada lordotik anormallik tespit edilmiştir. Ayrıca, 0,60 mg L^{-1} grubunda omurganın thoraco-abdominal birleşme yerinde deformasyon ve kaudal bölgede lordotik eğimde azalma görülmüştür. Balığın omurga dokusundaki kalsiyum ve potasyum seviyeleri 0,30 ve 0,60 mg L^{-1} phosalone konsantrasyonlarında önemli oranda azalmıştır. Omurga dokusundaki sodyum miktarı tüm phosalone konsantrasyonlarında, magnezyum ise 0,60 mg L^{-1} konsantrasyonunda önemli derecede azalmıştır. Phosalone kirliliği sucul ekosistemde omurga deformasyonlarını da içeren çeşitli olumsuz etkilere neden olabilir. Bundan dolayı phosalone, sucul ekosistemde kabul edilebilir değerlerin altında tutulmalıdır.

Anahtar Kelimeler: *Cyprinus carpio*, phosalone, toksisite, radyografi, mineral analizi.

Introduction

There are three main types of spinal column abnormalities: lordosis (ventral deformity, V shape), scoliosis (lateral deformity, zig-zig shape) and kyphosis (dorsal deformity, Λ shape) (Afonso *et al*., 2000). Spinal deformities have been described in many species of cultured fish such as Atlantic salmon

(*Salmo salar*) (McKay and Gjerde, 1986), rainbow trout (*Oncorhynchus mykiss*) (Aulstad and Kittelsen, 1971), carp (*Cyprinus carpio*) (Wunder, 1981), brown trout (*Salmo trutta*) (Poynton, 1987), sea bream (*Sparus aurata*) (Andrades *et al*., 1996; Afonso *et al*., 2000; Çoban *et al*., 2007; Saka *et al*., 2008) and sea bass (*Dicentrarchus labrax*) (Kayim *et al*., 2010). This can be a major problem on many fish farms and significantly influences the economical success of aquaculture. Generally, these abnormalities arise from the insufficient knowledge of optimum environmental preferences of fish at different stages of their lives (Messaoudi *et al*., 2009). Pesticides such as toluene, toxaphene, malathion, kepone, trifularin and pyrithione can cause abnormalities of morphological, skeleton and or spinal deformities in many fish living in fresh water (Weis and Weis, 1987; Lien *et al*., 1997; Mochida *et al*., 2008). Phosalone is an organophosphorus pesticide and insecticide used for protection of many vegetables and fruits against harmful predators. It is extremely toxic for fish and the other aquatic organisms and it contaminates the environment (Extoxnet, 2011).

Minerals are responsible for skeletal formation, maintenance of colloidal systems, regulation of acid-base equilibrium for biologically important molecules such as hormones and enzymes. Mineral deficiencies can cause biochemical dysfunction and structural deformities. These deficiencies depend on several factors such as the duration and degree of mineral deprivation (Watanabe *et al*., 1997).

Atikhisar Reservoir and Sarıçay Creek provide drinking and irrigation water for the city of Çanakkale, Turkey. In the study carried out by Kaya *et al.* (2010), excess levels of phosalone pesticide residue in Atikhisar Reservoir was detected. However, an investigation of the pesticide effects on the carp fish abundant in the reservoir is needed. In this experiment, the effects of different concentrations of phosalone on the morphological abnormalities (abnormal swimming), spinal deformities and some spinal mineral contents of carp fish were investigated.

Materials and Methods

Experimental Design

Juvenile common carp (n=144) average weighing 35.70±1.22 g (mean±SEM) were obtained from Mediterranean Fisheries Research Production and Training Institute, Beymelek, Antalya and acclimatized for 4 weeks in stock aquaria with flowing, aerated, and dechlorinated Çanakkale tap water (see below). The fish were fed a commercial carp diet containing 35.21% protein, 3.4% fat. Fish were divided among twelve 80 L experimental aquariums (12 fish/tank), in a triplicate design (3 tanks/treatment), and were allowed to rest for 24 h prior to the beginning of the experiment and they were not fed during this time to prevent contamination of the water with food debris. The fish were exposed to control (freshwater only), 0.15, 0.30 and 0.60 mg L^{-1} phosalone concentrations in triplicate to one of the following treatments for 14 days using a semi-static exposure regime (75% water change every morning, 25% every tonight with re-dosing after each change) (Smith *et al*., 2007). These sublethal concentrations were determined by considering LC_{50} doses (Nouyaku, 1989). Some water quality parameters were measured daily using YSI MPS 556 probe and water samples were taken for analysis after every water change. Values were; temperature, 23.27±0.12ºC; Salinity, 0.15‰; pH, 7.35±0.03; (measured by HANNA C 200, HI 83200 photometer) dissolved oxygen, 6.29±0.02 mg L^{-1}; total ammonia, 0.13±0.01 mg L^{-1} (measured by Thermo Aqumate VIS-Spectrophotometer). The electrolyte composition of the dechlorinated Çanakkale tap water used was 0.3, 0.04, 0.5 and 0.8 mmol L^{-1} for Na^+, K^+, Mg^+ and Ca^{2+} respectively (measured by Varian Liberty Sequential ICP-OES). Photoperiod was 12 h light: 12 h dark.

Phosalone Stock Solutions and Dosing

Analytical standard phosalone was obtained from Sigma–Aldrich (Steinheim, Germany, 99.5% purity). Phosalone, stored at +4°C, was prepared by weighing a defined amount and diluting it in acetone to prepare the stock material. The control group received the greatest volume of acetone used to dilute of the phosalone. The bioassay system was as described in standardized methods (APHA, AWWA, WEF, 1998).

Macroscopic and Radiographic Examination

Macroscopic and radiographic observations in fish were performed at the end of the study (14^{th} day). All the fish (36 fish/ each group) were macroscopically examined. The observed abnormal swimming fish were visually assessed and percentage of morphological abnormalities for each groups was calculated (Control, 0.15, 0.30 and 0.60 mg L^{-1}) (Mochida *et al*., 2008). The 0.30 mg L^{-1} and 0.60 mg L^{-1} groups with morphological abnormalities and the control group fish (6 fish/each groups) were anaesthetised with MS222 before killing and then they were placed directly onto the cassette and one-way radiograph was taken in laterolateral (LL) view. HOLOGIC LORAD M-IV type mobile X-ray apparatus was used, with the following parameters: tube voltage 22 kV, electronic time switch 45 mAs. The radiograph was printed on 11x14 size Konica Dry Pro 793 CR X-ray film. Later, mineral analysis of the fish was performed.

Spinal Tissue Mineral Analysis

At the end of the study (14^{th} day), fish

radiography taken (control, 0.30 and 0.60 mg L^{-1}) and the phosalone exposed of fish (0.15 mg L^{-1}, 6 fish) were used for tissue mineral analysis. Fish spinal tissue was carefully harvested. Tissues were oven dried and digested in 5 ml of concentrated nitric acid, then were diluted to 20 ml with deionised water and Na^{+}, K^{+}, Mg^{+} and Ca^{2+} were analysed using ICP-OES (Handy *et al*., 2000).

Statistical Analysis

Values were expressed as mean ± standard error of mean (SEM) for each parameter measured. Statistical assessment of results was carried out using SPSS 17 for Windows XP statistical software (Anonymous, 2011). Data sets were analyzed using Tukey post-test and Kruskal–Wallis one-way ANOVA by ranks followed by Dunn's post-test (Logan, 2010).

Results

During the experiment, while no death was observed for fish exposed to phosalone concentrations, abnormal swimming and lethargy were encountered at the medium (0.30 mg L^{-1}) and high doses (0.60 mg L^{-1}). The percentage of morphological abnormalities without categorization was given in Table 1. Percentage morphological abnormalities of 0.30 and 0.60 mg L^{-1} phosalone groups were significantly higher than the 0.15 mg L^{-1} phosalone and control groups ($P<0.05$) (Table 1). Lateral radiographs of a control and fish from treatment groups (0.30 and 0.60 mg L^{-1} phosalone concentrations) were shown in Figure 1 (A, B and C). Spinal deformity was observed in 0.30 mg L^{-1} and 0.60 mg L^{-1} fish treatments, which were radiographically examined. The radiographs showed that loss of kyphosis at the thoral spine (Figure 1, B1)

Table 1. Percentage morphological abnormalities and fish size in carp fish exposed to different concentrations of phosalone. [For parametric data the Tukey test was used $p<0.05$. Different letters in a column are significantly different. Values given as mean ±SEM]

Fish groups	N	Final fish weight (g)-Length (cm)	Concentration (mg L^{-1})	Morphological abnormalities (%)
Control	36	35.13±0.78-13.82±0.17	0.00	0
Low	36	35.43±0.66-13.12±0.17	0.15	0
Medium	36	36.04±1.22-13.83±0.52	0.30	35.33 ± 0.88^{b}
High	36	34.36±1.20-12.57±0.29	0.60	65.00 ± 1.15^{a}

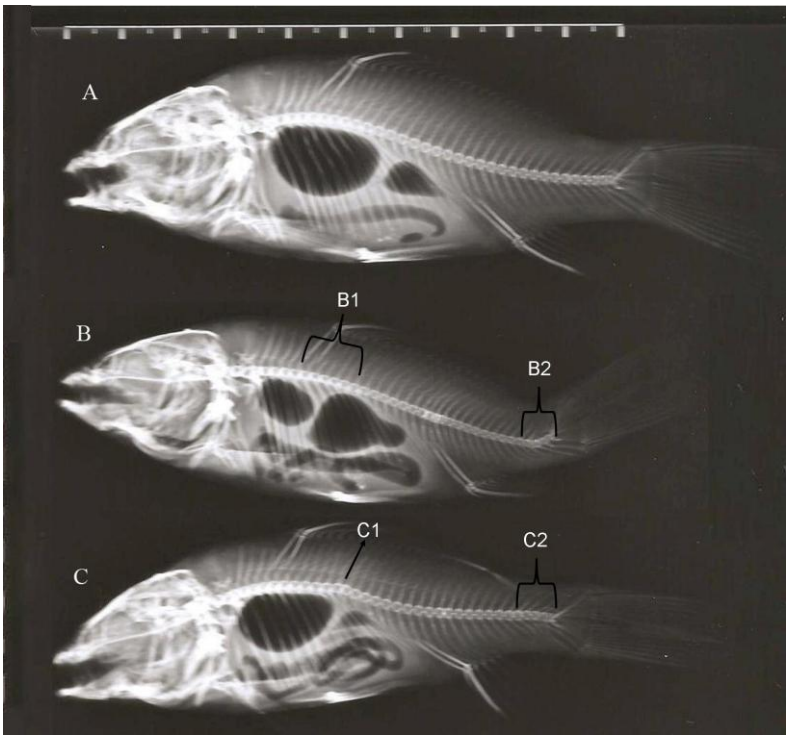

Figure 1. Radiography of lateral view of carp exposed to different phosalone concentrations.
A: Normal specimen, B1: Loss of kyphosis at the thoral spine (0.30 mg L^{-1}), B2: Severe lordotic anomaly at the caudal spine (0.30 mg L^{-1}), C1: Deformity at the thoraco-abdominal junction of the spine (0.60 mg L^{-1}), C2: Decrease of lordotic curvature in the caudal region (0.60 mg L^{-1})

and demonstrated a severe lordotic anomaly at the caudal spine (Figure 1, B2) in treatment group of 0.30 mg L^{-1} phosalone concentration compared to the control group. Deformity was observed at the thoraco-abdominal junction of the spine (Figure 1, C1) and the lordotic curvature decreased in the caudal region (Figure 1, C2) in the common carp exposed to 0.60 mg L^{-1} phosalone concentration compared to the control group.

The mineral content in the spinal tissue of carp fish were provided in Table 2. While the concentrations of Ca^{2+} and K^{+} in the treatment fish exposed to 0.30 and 0.60 mg L^{-1} of phosalone significantly decreased compared to the control group ($p<0.05$), the concentration of Mg^{2+} only decreased in the treatment fish exposed to 0.60 mg L^{-1} phosalone compared to the control group ($P<0.05$). Na^{+} concentrations in all treatment groups decreased compared to the control group ($P<0.05$). In terms of Ca^{2+}, Mg^{2+} and K^{+} mineral contents in the treatment fish exposed to 0.15 mg L^{-1} phosalone, the differences was non-significant compared to the control group ($P>0.05$).

Differences between the control and the treatment groups were not significant for fish weight and length ($P>0.05$) (Table 1-2). Abnormal swimming, lethargy and decrease in the food intake were observed in the treatment fish exposed to 0.30 and 0.60 mg L^{-1} of phosalone concentrations.

Discussion

In this study, two types of spinal deformities consisting of kyphosis and lordosis were determined (Figure 1). These basic deformities frequently occur in varying degrees of severity. Similary, some early studies have reported that exposure of malathion in *Heteropneustes fossilis* (Srivastava and Srivastava, 1990), *Brachydanio rerio* (Kumar and Ansari, 1984) and *Cyprinodon variegatus* (Weis and Weis, 1976), toxaphene in *Pimephales promelas* (Mehrle and Mayer, 1975) and carbaryl in *Oryzias latiped* (Solomon, 1977) caused skeletal or spinal deformities. In recent studies, it was reported that the malathion (Lien *et al*., 1997) copper pyrithione (Mochida *et al*., 2008) and zinc pyrithione (Sánchez-Bayo *et al*., 2005) showed skeletal or spinal deformities in *Clarias gariepinus*, *Fundulus heteroclitus* and *Oryzias latipes*, respectively. It was concluded that the occurrence of these deformities in the skeleton and spine could arise from decreasing amounts of collagen in the spinal column, changing amino acid composition (Mehrle and Mayer, 1975), deficiencies of vitamin C (Kumar and Ansari, 1984), neuromuscular spasms (Meiniel, 1981; Couch *et al*., 1977) and absence of a functional swimbladder (Chatain, 1994).

The present study showed that mineral concentrations in spinal tissue of fish exposed to phosalone in 0.30 and 0.60 mg L^{-1} was significantly decreased compared to the control group ($p<0.05$). This can be explained by the removal of the minerals from the osseous matrix. These vertebral changes may be caused by slight loss of structure causing collapse, fusion and alteration in intervertebral spacing resulting in various deformities (Eissa *et al*., 2009). It has been also reported that the vertebral changes cause demineralisation in fish. Various skeletal deformities including vertebral and spinal malformations are related to mineral deficiency (Lall, 2007). This was in agreement with another study that reported that the diameter and length of single vertebra were decreased in deformed fish and the mineral content was lower than normal fish (Baeverfjord *et al*., 1996).

0.30 and 0.60 mg L^{-1} phosalone concentrations caused morphological abnormalities, including lordosis or kyphosis in the spine of carp fish and decreased mineral concentrations in the spinal tissue. In conclusion, phosalone concentrations have to be kept under internationally accepted levels.

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Table 2. Fish size and mineral content in the spinal of carp fish exposed to different concentrations of phosalone (n=6). [Ca: Calcium, K: Potassium, Mg: Magnesium, Na: Sodium; for non-parametric data the Kruskal–Wallis test was used $P<0.05$). Different letters in a column are significantly different. Values given as mean ±SEM]

Fish groups	Concentration (mg L^{-1})	Final Fish Weight (g)-Length (cm)	Minerals (µg g^{-1})			
			Ca^{+2}	K^{+}	Mg^{+2}	Na^{+}
Control	0.00	36.48±0.66-13.15±0.40	10878.06±1768.50^{a}	4894.74±388.15^{a}	1245.69±271.79^{a}	3136.64±212.69^{a}
Low	0.15	35.48±2.77-12.03±0.62	8077.66±944.01^{ab}	3316.07±74.15^{a}	861.54±131.30^{a}	863.96±136.83^{b}
Medium	0.30	36.36±1.74-12.42±0.57	5876.50±869.16^{b}	1183.39±174.61^{b}	787.65±152.99^{a}	509.85±29.54^{b}
High	0.60	36.29±0.73-12.62±0.79	5073.80±681.00^{b}	1464.96±191.89^{b}	682.30±75.93^{b}	846.57±94.61^{b}

the manuscript.

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