EFEFCT OF KAOLIN ON GROWTH AND CLINICAL SIGNS IN COMMON CARP EXPOSED TO COPPER SULPHATE TOXICITY

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ABSTRACT

This study was aimed to investigate the effect of kaolin on growth and clinical signs in *Cyprinus carpio* exposed to copper sulphate. For this rationale, six treatment groups with two replicates were used in this experiment as follows: C- served as control negative group without exposed to kaolin or $CuSO_4$; C+ served as control positive group was exposed to 0.91mg/l $CuSO_4$ only; T1, T2, T3 and T4 were subjected to 0.91mg/l of $CuSO_4$ and kaolin was added to them at levels of 2, 4, 6 and 8 g/l respectively. Result findings reveal a positive relationship of the mean total weight gain among treatments T1, T2, T3 and T4 with the increase in the percentage of kaolin, practically in T4 which reached 13.2g. The mean daily weight gain of the experimental fish ranged between – 0.08 g/day/fish in C+ treatment to 0.55g/day/fish in C- treatment. C- treatment recorded the best value for feed conversion ratio and feed conversion efficiency with values of 3.5 and 28.57% respectively, followed by T4 with values of 3.9 and 25.0% respectively. In addition, values of the relative growth rates fluctuated between the lowest value in C+ treatment (-2.50%) and the highest value in C- treatment (18.34%). Specific growth rate, experienced the same previous pattern as they ranged between – 0.04 and 0.24%/day in C+ and C- treatments respectively. These results suggested that kaolin helped to reduce the toxicity of copper sulphate in experimental fish.

Keywords: fish, heavy metal, pollution, nutrition

تاثير الكاولين في النمو والعلامات السريرية لاسماك الكارب الشائع المعرضة لسمية كبريتات النحاس

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المستخلص

هدفت الدراسة التحري عن تأثير الكاولين في معدلات النمو والعلامات السريريه في اسماك الكارب الشائع المعرضة لكبريتات النحاس. استخدمت لهذا الغرض ست معاملات بواقع مكررين لكل معاملة وكالاتي: معاملة السيطرة السالبة بدون اضافة الكاولين أو كبريتات النحاس ومعاملة السيطرة الموجبة بتركيز 0,91 ملغم/لترمن كبريتات النحاس، تم تعريض المعاملات الاولى والثانية والثالثة والرابعة الى 0,91 ملغم/لترمن كبريتات النحاس وأضيف اليها الكاولين بنسب 2 و4 و6 و8 غم كاولين/لتر على التوالي. أظهرت النتائج وجود علاقة موجبة طردية لمعدل الزيادة الوزنية الكلية لأسماك التجربة مع زيادة نسبة الكاولين لاسيما في المعاملة الرابعة والتي بلغت 13,2 غم. تراوح معدل الزيادة الوزنية اليومية للأسماك بين – 0,08 غم/يوم/ سمكة في المعاملة +C إلى 0,55 غم/يوم/سمكة في المعاملة -C . سجلت المعاملة -C أفضل قيمة لمعدل التحويل الغذائي وكفاءة التحويل الغذائي بقيم 3,5 و 28,57% على التوالي، تلتها المعاملة الرابعة بقيم 3,9 و 25,0% على التوالي .تذبذبت قيم معدلات النمو النسبي بين أدنى قيمة لها في المعاملة +C(‎-2.50%‎) وأعلى قيمة لها في المعاملة -C (‎18.34%‎) ، واتخذت معدلات النمو النوعي النمط السابق نفسه اذ تراوحت بين – 0,04 – 0,24%/يوم في المعاملتين +C و -C على التوالي. يمكن ان نستنتج بان الكاولين ساعد في تخفيف سمية سلفات النحاس المعرضة لأسماك التجربة.

الكلمات المفتاحية: اسماك، معادن ثقيلة، تلوث، تغذية

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INTRODUCTION

Pollution in aquatic environments significantly affects fish health, and the common carp *Cyprinus carpio* is often used as a model species to study these impacts **,** this globally distributed fish is highly resilient and adaptable, making it a reliable bioindicator of water quality (11,30). Pollutants such as heavy metals, pesticides, and industrial waste can accumulate in the tissues of common carp, leading to physiological stress, immune suppression, and behavioral changes (35). Common carp, is a globally important fish found in Eastern Europe and Central Asia, it is the world's most common freshwater fish species (3,6) .Their ability to establish quickly in newly acquired environments was linked to life history traits including fast development, early maturity, and high reproductive capability (35).Heavy metals are continually released into the aquatic environment from natural processes such as volcanic activity, weathering of rocks and industrial processes (7,8). Copper (Cu) is an essential microelement for living organisms, chloroplast reactions, enzyme systems related to photosystem (4).Copper, is highly toxic metal that is often considered poisonous even at low concentrations, is responsible for numerous metabolic processes are required to biological processes, introduced to water bodies from industrial waste or use of copper sulphate $CuSO_4.5H_2O$ as therapeutic or algaecide agent (22,31). Toxicity of Cu is related to gill dysfunction and sodium loss, respiration stress and oxidative stress (17).Copper salts (copper hydroxide, copper carbonate and copper sulphate) are widely used in agriculture as fungicide, algaecide and nutritional supplement in fertilizers (22,27).In the absorption process there are four possible routes for metals to enter a fish: the food ingested; simple diffusion of the metallic ions through gill pores; through drinking water; and by skin adsorption (17,26).The primary target of copper sulphate toxic action on fish is gills and then spread to liver and kidney caused abnormal behavior, histological changes and may be rapid death (4).Many studies have indicated that there are several materials or methods through which heavy metals can be removed from water, such as_zeolite (1,21,24) and kaolin (12,13, 25,33), as well as treated against bacteria (16). Kaolin Al_2O_3 $*4_2SiOn*2H_2O_5$ is a clay mineral made up of material that has been extensively used in a variety of technological uses , due to population increased , industrial manufacturing companies are expanding globally , and the wastewater produced by these industries has been discharged into lakes and rivers without adequate care (18,22,29). Kaolin, a natural clay mineral, is widely recognized for its adsorption properties and has a high surface area and negatively charged particles that attract and bind positively charged copper ions (Cu^{2+}) (18). This binding capacity reduces the bioavailability of harmful compounds such as copper sulphate, thus protecting the gut lining and promoting normal digestive function (3). Adding kaolin in experimental treatments, could led to reduce the accumulation of copper sulphate, particularly in gills (4,9). Kaolin also provides an inexpensive treatment for a costly fish disease because plentiful clay is available in Iraq(3). Due to of its adsorbent ability, kaolin as applied in hatchery processes in aquaculture to reduce egg adhesion as well as added to fish diets to prevent enteric diseases via its capability to absorb enterotoxins and major mycotoxins(20,28). However, these properties suggest that the effectiveness of kaolin at heavy metals and microbial binding may vary across disparate water chemistries and environmental conditions; thereby necessitating testing on a case-by-case basis before implementation in a culture setting (20). The present study was undertaken to investigate the effect of kaolin on growth and clinical sings in common carp exposed to copper sulphate.

MATERIALS AND METHODS

Preparing of kaolin clay: The natural white kaolin , untreated with any chemical and under suitable preservation conditions, was obtained from the Ministry of Industry and Mineral / Geological Survey / Department of Mineral Extraction, it was cleaned from dust in the laboratory, then small parts were taken and grinding with a grain mill for the purpose of obtaining kaolin powder, then sterilized well using the oven at the temperature of 90 ^{0}C for 6h.(3).

Experimental fish and design

A total of 120 fingerlings of common carp weighing 85-95g., with no visible signs of disease or morbidity were used in this experiment. Fish were acclimated to laboratory conditions for 14 days before beginning of the experiment. Glass aquariums used in the experiment, with dimensions of 70×40×40cm, using de-chlorinated tap water filled the aquarium to reach 50L and enough oxygen supply, no amount of water was changed during the experiment period. Fish were divided into six treatment groups with two replicates as follows: C- served as control negative group without exposed kaolin or $CuSO_4$: C+ served as control positive group exposed to 0.91mg/l$CuSO_4$ only; T1, T2, T3 and T4 were exposed to 0.91mg/l of $CuSO_4$ and kaolin was added to them at levels of 2, 4, 6 and 8 g/l respectively. These levels of kaolin were selected according to study of Beck et al. (9). All treatments were fed daily at 3% body weight. After 30 days of experimental trail, fish were weighed and recorded survival rate. Chemophysical characteristics of the water were recorded as follows; temperature ranged between 24 – 26 °C, pH values ranged between 7.7–8.2, while dissolved oxygen level ranged between 5.4 – 7.5 mg/l . Additionally, salinity in water ranged from 0.05 to 0.08 g/l. These values within the natural limits of live for warm fish particularly common carp (35).

Growth performance: Body weight gain = Mean final weight Kaolin, a natural clay mineral, is widely recognized for its adsorption properties and has a high surface area and negatively charged particles that attract and bind positively charged copper ions (Cu^{2+}) (18). This binding capacity reduces the bioavailability of harmful compounds such as copper sulphate, thus protecting the gut lining and promoting normal digestive function (4). Adding kaolin in experimental treatments, could led to reduce the accumulation of copper sulphate, particularly in gills (9,10). Mean initial weight

Daily weight gain = $W_2 - W_1 / T$

W_2 = Mean final weight, W_1= Mean initial weight, (T) = Duration of experiment

Feed conversion ratio (F.C.R.) =Total feed consumed by fish / Total weight gain by fish

Feed conversion efficiency (F.C.E.) =Total weight gain by fish / Total feed consumed by fish ×100

Relative growth ratio (R.G.R.) = Final weight –Initial weight / Initial weight× 100.

Specific growth rate (S.G.R.% day) = Ln of final weight (LnW_2) - Ln of initial weight (LnW_1) / Duration of experiment (T) ×100.

Survival rate = Final number of fish / Initial number of fish x 100 (37).

Behavioral changes

Clinical sings of fish were recorded included swimming, respiration, feed intake, reaction to external stimuli and death.

Statistical analysis

The influence of various factors on research parameters was determined using the SAS software, or Statistical Analysis System. In this study, the least significant difference test (ANOVA) was employed to compare means in a meaningful way. P value was considered significant difference lees than 0.05.

RESULTS AND DICUSSION

Weight indices: The results showed that fish exposed to copper sulphate without kaolin (C+ treatment) experienced weight loss, with mean total weight gain ranging from -2.4g to 16.6g, it may be due to the loss of appetite for intake feed in this treatment, while a positive correlation was observed between kaolin percentage and weight gain, particularly in T4, which verified a mean total weight gain of 13.2g. The mean daily weight gain ranged from -0.08g/day/fish in C+ to 0.55g/day/fish in C- treatment, with improved daily weight gain observed as kaolin levels increased. Statistical analysis showed significant differences ($p \leq 0.05$) in C- treatment compared to other treatments (for final weight, total weight gain, and daily weight gain). While, no significant differences ($p > 0.05$) were observed among T2, T3, and T4 for these parameters. These findings confirm that increasing the level of kaolin effectively mitigates the toxic impacts of copper sulphate, enhancing the growth parameters of the experimental fish (Table1).These findings demonstrate the significant role of kaolin in reducing the toxic effects of copper sulphate on fish and promoting their growth performance. Exposure to copper sulphate alone (C+ treatment) led to weight loss, indicating its adverse impact on fish health and metabolic processes. The observed weight reduction aligns with findings in previous studies that highlight the harmful effects of heavy metals like copper on aquatic organisms, including disturbance of biochemical pathways and physiological processes (10,32,40). The introduction of kaolin, however, revealed a clear protective effect, as demonstrated by the enhancement in weight gain with increasing kaolin levels, particularly in T4. Kaolin likely acts by binding to copper ions, reducing their bioavailability and toxicity (3). This mechanism supports previous research that detects kaolin as an effective adsorbent for heavy metal detoxification (3,4). The significant weight gain in kaolin-treated fish underscores its potential to enhance recovery and growth under toxic conditions (9). The lack of significant differences in weight parameters among T2, T3, and T4 suggests that beyond a certain threshold, the effect of kaolin on copper detoxification stabilizes, indicating an optimal level for kaolin application (9). This result is important for practical applications in aquaculture, as it offers guidance on the effective dosage for mitigating copper sulphate toxicity without excessive use. As it is known, some of the adverse effects of heavy metal pollution arise from their inhibitory effects on enzyme metabolism in common carp, besides that, it is also known that the cholesterol, lipoproteins, and triglyceride values are connected with the metabolism of lipids and functions of liver and kidney (38). Uncumusaoğlu (36) mentioned that the copper sulphate caused the biggest effect on weight gain in *C. carpio* using fed different levels of copper sulphate.

Table 1. Weight indices (Mean ± SE) for *C. carpio* with different concentrations of kaolin exposure to copper sulphate

Wt. indices / Treatment	Initial weight (g)	Final weight (g)	Total weight gain (g)	Daily weight gain (g/day/fish)
C –	90.5 ± 0.58^{a}	107.1± 3.2^{a}	16.6 ± 3.2^{a}	0.55 ± 0.02^{a}
C+	92.7 ± 1.58^{a}	90.3 ± 2.2^{d}	-2.4 ± 0.02^{d}	-0.08 ± 0.03^{d}
T1	88.4 ± 0.46^{a}	95.8 ± 2.1^{c}	7.4 ± 0.7^{c}	0.24 ± 0.03^{c}
T2	89.5 ± 0.57^{a}	99.0 ± 1.9^{b}	9.4 ± 0.5^{b}	0.31 ± 0.02^{b}
T3	88.7 ± 1.20^{a}	101.4 ± 1.8^{b}	12.7 ± 0.6^{b}	0.42 ± 0.02^{b}
T4	88.6 ± 0.95^{a}	101.8 ± 1.5^{b}	13.2 ± 0.6^{b}	0.44 ± 0.02^{b}

Different alphabetic letters indicated significant variations at ($p \leq 0.05$).

Feeding indices: The results in Table 2 indicate that the C- treatment recorded the best performance in terms of feed conversion ratio (FCR) and feed conversion efficiency (FCE), with values of 3.5 and 28.57%, respectively. This was followed by the T4 treatment, which achieved FCR and FCE values of 3.9 and 25.0%, respectively. The T3 treatment exhibited results close to T4, with FCR and FCE values of 4.0 and 25.0%, respectively. In contrast, the C+ treatment showed the lowest performance, with an FCR of 5.0 and a negative FCE value of -20.0%. Statistical analysis revealed significant differences ($p \leq 0.05$) in the C+ treatment compared with the other treatments in terms of both FCR and FCE. However, no significant differences ($p > 0.05$) were observed among T1, T2, T3, and T4 in FCR. Results indicated that is a negative relationship (in value) between feed conversion ratio and feed conversion efficiency (10,21).It may be attributed to the accumulation of copper sulphate tissues of the experimental fish led to loss the appetite in fish , followed by an increase in the value of food conversion ratio with large amounts of uneaten food remaining at the bottom of the aquariums in this treatments, this results agree with Ramos-Misra(34) who indicated that the copper sulphate accumulated in tissues particularly in gills, blood, kidneys, intestine and liver.The role of kaolin in mitigating copper sulphate toxicity is particularly noteworthy. Treatments like T4 and T3, which included kaolin supplementation, demonstrated improved FCR (3.9 and 4.0, respectively) and FCE (51.0% for both), suggesting that kaolin effectively mitigated the adverse effects of copper sulphate. In contrast, the C+ group, which showed the highest FCR of 5.0 and a negative FCE (20.0%). This is in line with previous findings that excessive copper sulphate exposure disrupts gut integrity and enzyme activity, leading to poor nutrient absorption and energy losses (32). The statistical significance ($p \leq 0.05$) of the differences between the C+ and other treatments underscores the detrimental impact of copper sulphate toxicity and the protective role of kaolin. Interestingly, the absence of significant differences ($p > 0.05$) among treatments T1, T2, T3, and T4 in FCR indicates that kaolin supplementation levels within these treatments may have reached an effective threshold, minimizing variability in feed efficiency outcomes. This finding aligns with the observation of Al-Ghazaly and Al-Rudiany (3), who stated that even low to moderate levels of kaolin could neutralize gastrointestinal toxins and enhance feed efficiency in fish, which suggest that when feed compositions are balanced and nutrients are adequate, performance differences can become less pronounced (34). The accumulation of metal such as copper sulphate in different parts of the fish body depends on many factors such as feeding behavior, water quality, fish species , age, size, reproductive stage, fish health, bioavailability of the metal, and variations between habitats (20,34).

Table 2. Feeding indices (Mean ± SE) for *C. carpio* with different concentrations of kaolin exposure to copper sulphate

Feeding indices / Treatment	F.C.R.	F.C.E.(%)
C –	3.5 ± 0.6[c]	28.00 ± 3.0[a]
C+	5.0 ± 0.9[a]	- 20.00 ± 1.0[d]
T1	4.5 ± 0.7[b]	22.22 ± 3.0[c]
T2	4.2 ± 0.7[b]	23.80 ± 2.5[c]
T3	4.0 ± 0.6[b]	25.00 ± 2.6[b]
T4	3.9 ± 0.5[b]	25.64 ± 2.4[b]

Different alphabetic letters indicated significant variations at ($p \leq 0.05$)

Growth indices: The results of the growth performance, presented in Table 3, revealed that different concentrations of kaolin had varying effects on fish growth, with the most notable changes observed in T4. A significant reduction in growth occurred in the C+ treatment, where fish were exposed to copper sulphate alone, resulting in the lowest relative growth rate (RGR) of -2.50%, while the highest RGR of 18.34% was recorded in the C- treatment. In treatments T1 to T4, RGR values ranged from 8.37% to 14.89% respectively. Similarly, the specific growth rate (SGR) ranged from -0.04%/day in the C+ treatment to 0.24%/day in the C- treatment, increasing with kaolin concentration from 0.12%/day in T1 to 0.20%/day in T4. Survival rates showed stark contrasts, with no mortality in the C- treatment (neither copper sulphate nor kaolin), while the C+ treatment (copper sulphate only) led to the death of all fish within three days. The results of the statistical analysis indicate a significant increase ($p \leq 0.05$) in C- treatment compared to the other treatments for relative growth and specific growth rates. No significant differences ($p > 0.05$) appeared between T1 and T2 , and between T3 and T4 when referring to the relative growth and specific growth rates. As a result, a significant reduction in growth was observed in the fish exposed to this metal, particularly in the C+ treatment. Fish growth serves as an indicator of population health, and can be used to detect stress caused by contamination (19,24). Cu is used directly in biological processes such as mitochondrial respiration and it actively participates in free radical detoxification (5,14,34). The results of this study highlight the significant impact of copper sulphate and kaolin on the growth performance and survival of fish. The observed decreased in growth, particularly in the C+ treatment, can be credited to the toxic impacts of copper sulphate, which is known to interfere with vital physiological processes in aquatic organisms, leading to poor growth and high mortality. The complete mortality of fish within three days in the C+ treatment underscores the acute toxicity of copper sulphate when used alone, even at the concentration applied in this study. This observation is in line with Al-Tamimi and Al-Rudainy (4) and Harikrishnan et al. (22). In treatments involving kaolin (T1 to T4), the results indicate a concentration-dependent improvement in growth metrics, such as RGR and SGR, with increasing kaolin concentrations. This proposes that kaolin may offer a protective effect against copper sulphate toxicity by adsorbing or neutralizing its harrmful components, thereby mitigating its impact on fish (4). However, the protective effect was not sufficient to fully counteract the negative impacts of copper sulphate, as growth metrics in these treatments were still lower than the C- treatment. Additionally, Kaolin supplementation in animal diets has been shown to protect animals against diarrhea (3), as well as aflatoxins, pathogenic microorganisms, and heavy metals (2,11). When humans consume contaminated fish or come into direct contact with these toxins, negative health effects may occur, including poisoning, respiratory problems, or skin rashes (4). As an immune stimulant, kaolin has been found to be safe for fish health (23). Beck et al. (9) demonstrated that kaolin adsorbs bacteria and metals binds to them, preventing their attachment to fish and thereby reducing the disease burden. Additionally, kaolin helps decrease the presence of organic matter and microbes. It also significantly reduces the adhesion of metals to healthy catfish mucus, with this effect becoming more pronounced at higher concentrations (23).

Table 3. Growth indices (Mean ± SE) for *C. carpio* with different concentrations of kaolin exposure to copper sulphate

Growth indices Treatment	R.G.R (%)	S.G.R. (%day)	Mortality	Survival rate (%)
C –	18.34 ± 1.2^{a}	0.24 ± 0.1^{a}	0	100
C+	-2.50 ± 2.1^{d}	- 0.04 ± 0.1^{d}	10	00
T1	8.37 ± 1.1^{c}	0.12 ± 0.0^{c}	5	95
T2	10.61± 1.3^{c}	0.15 ± 0.0^{c}	4	96
T3	14.13 ± 0.9^{b}	0.19 ± 0.0^{b}	2	98
T4	14.89 ± 0.7^{b}	0.20 ± 0.1	2	98

Different alphabetic letters indicated significant variations at ($p \leq 0.05$).

Clinical signs and behavioral changes

The clinical signs observed in fish exposed to copper sulphate (C+ treatment) indicate significant clinical and behavioral distress. These included loss of scales, eye abnormalities, pectoral fin rot, hemorrhage, and darkening of the skin, along with erratic swimming behavior characterized by imbalance and flashing. Such signs are consistent with acute stress responses and toxic effects induced by heavy metal exposure, as copper sulphate is known to cause oxidative stress and tissue damage in aquatic organisms. These results align with existing studies (15,39), which have documented similar pathological changes in fish subjected to heavy metal toxicity. Conversely, abnormal clinical signs and behavior were noted in groups treated with kaolin, particularly in T4, followed by T3 and T2, as well as in the C- treatment. Kaolin, while not directly toxic, may affect fish through indirect means such as mechanical irritation or interference with normal gill function, especially at higher concentrations or prolonged exposure, despite these effects, kaolin has shown therapeutic potential in veterinary practices, particularly in managing gill infections (3). This observation aligns with findings by Beck et al. (9), who reported the efficacy of kaolin on channel catfish, infected with columnaris disease in reducing bacterial attachment to target tissues.

CONCLUSION

This study confirms the efficiency of kaolin in mitigating copper toxicity and promoting fish health, offering valuable insights for aquaculture management. Combining kaolin in systems exposed to heavy metals could improve productivity and reduce the risks and toxic effects associated with copper pollution. Reduction or removal of some emerging pollutants is more obstinate and requires the development of novel remediation projects.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

DECLARATION OF FUND

The authors declare that they have not received a fund.

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