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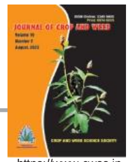

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Bioaccumulation of heavy metals in *Pangasius hypopthalamus* cultured in sewage treated water

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ABSTRACT

The current research was designed to scrutinize the bioaccumulation of heavy metal (Cu, Mn, Fe, Zn) content in different organs of Pangasius hypopthalamus cultured in sewage treated water. The levels of heavy metals varied significantly among fish muscle, stomach, gills, heart and brain tissue. The gills were the target organ for the manganese (3.377 mg kg^{-1}), iron (8.46 mg kg^{-1}) and zinc (1.276 mg kg^{-1}) accumulation, whereas, maximum copper concentration was found in muscle (2.013 mg kg^{-1}) followed by gills (0.296 mg kg^{-1}). The bioaccumulation of heavy metals was maximum in gills succeeded by muscle, brain and heart. Least amount was bio-accumulated in stomach. The net accumulation of iron was maximum in organs followed by manganese, copper and zinc. Nickel and chromium were undetectable in sewage treated water. A remarkable variation in the deposition of elements in various organ of fish species was reported statistically (p =0.05).

Keywords: Bioaccumulation, fish organs, heavy metals, *Pangasius hypopthalamus,* sewage treated water

The fisheries sector contributes to employment and income generation as it accelerates the development of numerous subsidiary industries and additionally meets the hunger demand of the growing population as it is a source of cheap and nutritious food (Ghosh *et al.*, 2016). However with rising population, water quality is degrading constantly which affects the aquatic life. Metal concentrations in aquatic organisms such as fish and shell fish are considerably higher than in water or sediments because these organisms tends to accumulate metal through their metabolism (Olaifa *et al.*, 2004). They can readily uptake metals and have varying levels of metal accumulation in different tissues (Khaled, 2004). In order to ensure enzyme activity and many other biological processes, it is essential to keep some heavy metals at low levels, such as cobalt, zinc, copper, manganese and iron, as at high levels, they can be quite toxic (Bryan, 1976). Heavy metals like mercury, lead and cadmium are toxic even at low concentrations, though it is unclear what role they play in living organisms. The absorption of heavy metals, the effect that heavy metals have on the fish, and the sensitivity of the organism to heavy metals extremely vary depending on the concentration of heavy metals, the fish's age, size, physiological state, habitat preferences, feeding habits, and growth rate (Zhang *et al.*, 2007). In addition to affecting the aquatic environment, heavy metals have an abiding effect on living organisms, especially these non-biodegradable metals accumulated in human, animal, and plant organs (Saad *et al*., 2012). Histopathological studies showed that as a result of heavy metal contamination of water, organs and muscle composition were damaged (Tayel *et al*., 2013).

Treated wastewater is becoming popular as propitious avenue of livelihood generation through its use for aquaculture (Roy *et al*., 2011; Cuevas-Uribe and Mims, 2014) as it can curtail the demand for freshwater and input costs in aquaculture operations, thereby maximizing profitability. However, sometimes treated wastewater's quality is suboptimal due to presence of heavy metals and other hazardous compounds. Fishes are highly vulnerable to water pollution due to bioaccumulation of toxic chemicals from continuous and prolonged exposure to contaminated water (Dutta *et al*., 2022). There is a good possibility that heavy metals will enter aquatic organisms as they dissolve easily in aqueous medium (Das *et al*., 2007; Dutta *et al*., 2022). Human health can be compromised even if a fish at a lower trophic level consumes even smaller concentrations of heavy metal repeatedly. A fish's gills, skin, and gastrointestinal tract contain epithelium and mucosa that readily absorbs heavy metals (Jovanoviæ *et al*., 2011). Using data from a sewage-fed aquaculture, Darko *et al.* (2016) evaluated the health risks associated with toxic metal consumption, finding mercury to pose the greatest risk, and lead to pose the lowest. Keeping these points in view, the bioaccumulation of heavy metals in *Pangasius hypopthalamus* tissues cultured in sewage treated water was evaluated in the present study.

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MATERIALS AND METHODS

For present study, sewage treated water was collected from Sewage treated water ponds (Fig. 1) located near Vegetable Research Farm, CCSHAU, Hisar and brought to the water quality laboratory of the College of Fisheries Science, CCSHAU, Hisar. The water was filtered to remove the twigs and debris before pouring into the glass aquarium (size 60 × 18 inches base, 24 inches height, 12 mm thick glass). The water samples from sewage treated water ponds were collected on 0 day in triplicate to analyze the presence of six heavy metals viz., zinc (Zn), manganese (Mn), iron (Fe), copper (Cu), nickel (Ni) and chromium (Cr) before releasing *P. hypopthalamus.* Prior to analysis, Whatman filter paper was used to filter the water samples. Advance Fingerling of *P. hypophthalamus* (1.76 to 6.1 g and 6.02 to 10.10 cm) was released into the sewage treated water under triplicate conditions, each replicate cultured 20 fishes with four aerators. A double daily feeding of 8 percent of their body weight was administered to the fish. For the collection and preparation of fish tissue samples, *P. hypopthalamus* samples before and after three months of release were collected under penta-replicate condition from experimental aquariums. The muscle tissue, stomach, gills, heart and brain of fishes were dissected and cleaned with distilled water. The tissues were kept in separate poly bags (Fig. 2) in deep freezer at -4 ^{0}C until analysis. For heavy metal analysis, fish tissue samples were prepared using the wet digestion method of Chernoff (1975). After thawing and homogenizing the frozen fish samples, acid digestion was performed. To 2 g of homogenized tissue sample, 5 ml concentrated HNO_3 was added and incubated at a temperature around 90 ± 0.5°C in water bath. After 5 min, 5 ml of concentrated H_2SO_4 and $HClO_4$ solution (3: 2) was added gradually. After digestion, the samples yielded a pale transparent solution. The digested samples were cooled down, diluted and adjusted to the desired final volume of 20 ml with distilled water. Filtration was performed using Whatman filter paper 40 for the final solution. A flame atomic absorption spectrophotometer (FASS) was used to analyse the water samples and processed fish tissue for heavy metals. The readings obtained were multiplied by the dilution factor. Bioaccumulation factor for different organs of fish was calculated to check the net bioaccumulation of heavy metals in fish tissues. Using mg kg^{-1} as a unit, the individual heavy metal concentrations were calculated.

$$\text{Bioaccumulation factor (B.F.) (L kg}^{-1}\text{)} = \frac{\text{Heavy metal concentration in fish tissue (mg kg}^{-1}\text{)}}{\text{Heavy metal concentration in water sample (mg l}^{-1}\text{)}}$$

Statistical Analysis: Two factorial ANOVA was used to analyse concentration of heavy metal in *P. hypopthalamus* tissues reared in sewage treated water.

RESULTS AND DISCUSSION

The mean value of copper, manganese, iron and zinc in sewage treated water samples were 0.04, 0.008, 0.024 and 0.02 mg ml^{-1}. There was no detectable level of nickel and chromium in these samples.

Uptake of Copper (Cu) by Pangasius hypopthalamus

The uptake of copper ions by fish's muscle, stomach, gills, heart and brain in sewage treated water are expressed in terms of its concentration in Table 1. The data generated over the period of study exhibited significant increase (0.822 mg kg^{-1}) in the Copper concentration in fish organs after 90 days (CD=0.015; p=0.05) as compared to 0 day (0.260 mg kg^{-1}). Fish muscle tissue recorded the highest Cu concentration (2.01 mg kg^{-1}), succeeded by gills (0.296 mg kg^{-1}), stomach (0.177 mg kg^{-1}) and heart (0.114 mg kg^{-1}). Least Cu concentration (0.107 mg kg^{-1}) was observed in brain which was statistically comparable with its concentration in heart (CD=0.024; p=0.05). Interaction between Cu concentration in fish organs and duration was significant showing significantly higher concentration in all organs at 90 day than at 0 day (CD=0.034; p=0.05). In infants and children, copper deficiency may impair neurological and immunological abilities, cardiovascular development and can lead to bone malfunction (Gambling and McArdle, 2004; Georgieff, 2007). Moreover, in adults it may alter cholesterol metabolism (Reiser *et al*., 1987). However, excess copper leads to oxidative cell damage and cell death. According to Hidaka, 1970; Sutterlin and Sutterlin, 1970; Iwasaki and Sato, 1984, copper inhibits gustatory activities in carp, salmon, and mouse respectively. Copper concentrations estimated in the fish tissues were within the WHO (2011) permissible limit (3 mg kg^{-1}). Jovanoic *et al.* (2017) estimated similar results where the muscle of the carnivorous catfish was reported with highest content of Copper (Cu) metal (1.55 to 1.62 mg kg^{-1}).

Uptake of Manganese (Mn) by Pangasius hypopthalamus

Consistent with the results above, significant increase in concentration of manganese ions was found in fish organs at 90th day (1.695 mg g^{-1}) (CD=0.003; p=0.05) as compared to 0 day (Table 2). There was significantly higher manganese concentration in the gills of the fish gills (3.377 mg kg^{-1}) at the end of the

Table 1: Copper uptake by *Pangasius hypophthalmus* organs in sewage treated water

Fish organs	Copper concentration (mg kg^{-1})		Mean
	0 day	**90th day**	
Muscle	0.746	3.280	**2.013**
Stomach	0.146	0.208	**0.177**
Gills	0.222	0.370	**0.296**
Heart	0.098	0.130	**0.114** [a]
Brain	0.090	0.124	**0.107**[a]
Mean	**0.260**	**0.822**	

Values denoted by similar letter do not differ significantly with each other
CD (p=0.05) for Fish organs =0.024; SE(m) = 0.008
CD (p=0.05) for duration=0.015; SE(m) =0.005
CD (p=0.05) for Fish organs × duration =0.034; SE(m) = 0.012

Table 2: Manganese uptake by *Pangasius hypophthalmus* organs in sewage treated water

Fish organs	Manganese concentration (mg kg^{-1})		Mean
	0th day	**90th day**	
Muscle	0.838	1.108	**0.973**
Stomach	0.704	0.796	**0.750**
Gills	1.934	4.820	**3.377**
Heart	0.724	0.856	**0.790**
Brain	0.750	0.894	**0.822**
Mean	**0.990**	**1.695**	

CD (p=0.05) for Fish organs =0.004; SE(m) = 0.001
CD (p=0.05) for duration=0.003; SE(m) =0.001
CD (p=0.05) for Fish organs × duration =0.006; SE(m) = 0.002

Table 3: Iron uptake by *Pangasius hypophthalmus* organs in sewage treated water

Fish organs	Iron concentration (mg kg^{-1})		Mean
	0 day	**90th day**	
Muscle	2.500	9.200	**5.850**
Stomach	1.780	1.802	**1.791**[a]
Gills	2.900	14.020	**8.460**
Heart	0.954	1.356	**1.155** [a]
Brain	0.868	5.480	**3.174**
Mean	**1.800**	**6.372**	

Values denoted by similar letter do not differ significantly with each other
CD (p=0.05) for Fish organs =0.067; SE(m) = 0.022
CD (p=0.05) for duration=0.042; SE(m) =0.014
CD (p=0.05) for Fish organs × duration =0.095; SE(m) = 0.032

investigation period compared with the muscle (0.973 mg kg^{-1}), brain (0.822 mg kg^{-1}), heart (0.790 mg kg^{-1}) and stomach (0.750 mg kg^{-1}) (CD=0.003; p=0.05). Interaction between Mn concentration in fish organs and duration was significant (CD=0.006; p=0.05) showing significant accumulation of manganese (4.820, 1.108, 0.894, 0.856, 0.796 mg kg^{-1}) at 90th day than at 0 day (1.934, 0.838, 0.750, 0.724, 0.704 mg kg-1) in gills, muscle, brain, heart and stomach, respectively. In lakes of north-western Poland, Rajkowska and Protasowicki (2012) found that pike gills contain 2.0-2.3 mg kg^{-1}, muscle contains 0.2 mg kg^{-1}, and the digestive tract contains 0.7-0.8 mg kg^{-1} of manganese. The estimation of heavy metals from the present study were also

Table 4: Zinc uptake by *Pangasius hypophthalmus* organs in sewage treated water

Fish organs	Zinc concentration (mg kg^{-1})		Mean
	0th day	90th day	
Muscle	1.298	1.700	**1.499**
Stomach	0.730	0.802	**0.766**
Gills	0.838	1.714	**1.276**
Heart	0.436	0.570	**0.503**
Brain	0.430	1.072	**0.751**
Mean	**0.746**	**1.172**	

CD (p=0.05) for Fish organs =0.039; SE(m) = 0.013
CD (p=0.05) for duration=0.025; SE(m) =0.008
CD (p=0.05) for Fish organs × duration =0.055; SE(m) = 0.019

Table 5: Bioaccumulation of heavy metals in fish cultured in sewage treated water

Fish organs	Heavy metal bioaccumulation in fish cultured in sewage treated water (L kg^{-1})							
	Copper		Manganese		Iron		Zinc	
	0 day	90 day	0 day	90 day	0 day	90 day	0 day	90 day
Muscle	186.50	410.00	104.75	138.50	104.16	383.30	64.90	85.00
Stomach	36.50	52.00	88.00	99.50	74.16	75.00	36.50	40.10
Gills	55.50	92.50	241.75	602.50	120.83	584.50	41.90	85.70
Heart	24.50	25.00	90.50	107.00	39.75	56.50	21.80	28.50
Brain	22.50	31.00	93.75	111.75	36.16	228.33	21.50	53.60

supported by Osman *et al*. (2010) whose results showed a similar range (0.260-3.601 mg kg^{-1}) of manganese in the flesh of two species (*Tilapia zillii* and *Oreochromis niloticus*) collected from El-Serw Fish Farm situated on the shore of Lake Manzalah in Northeast Cairo (Egypt).

Uptake of Iron (Fe) by Pangasius hypopthalamus

A significant variation of iron concentration was observed in all the tissues of *P. hypophthalmus* (Table 3). On 90th day, significantly higher concentration (6.372 mg g^{-1}) was recorded than at 0 day (1.800 mg kg^{-1}) (CD=0.042; p=0.05). Among the five tissues, the Fe concentration in gills was observed maximum (8.460 mg kg^{-1}) and in the heart minimum (1.155 mg kg^{-1}) which was statistically comparable with iron concentration in stomach (1.791 mg kg^{-1}) (CD=0.067; p=0.05). The concentration of iron recorded in brain and muscle was 3.174 and 5.850 mg kg^{-1}, respectively. The interaction between Fe concentration in fish organs and duration was significant (CD=0.095; p=0.05) depicting significant differences in iron concentration in tissues at 0 and 90th day. Iron being an essential element in human diet forms an important part of haemoglobin allowing to carry oxygen from the lungs to the tissues. Humans experience anaemia as a result of severe Fe deficiency. As estimated in the present study, the iron content ranged from 1.155 to 8.460 mg kg^{-1} in different organs of P. *hypophthalmus* which is within the permissible limit (100 mg kg^{-1}) by WHO (2011). Akan *et al*. (2012) found out that *Synodentis budgetti* gills contained the highest concentration of Fe ranging from 0.68 to 12.65 µg g^{-1} while *Oreochronmis niloticus* flesh contained the lowest concentration at 0.68 µg g^{-1} which were similar to the study findings. Rajkowska and Protasowicki (2012) reported Fe content in the range of 0.8 to 93.4 µg g^{-1} and 1.4 to 87.7 µg g^{-1} in the organs of pike (*Esoxlucius* L.) obtained from the two lakes of North western Poland, respectively.

Uptake of Zinc (Zn) by Pangasius hypopthalamus

Observations on the Zinc concentration before (0.746 mg kg^{-1}) and after the termination of experiment are presented in Table 4. It showed higher concentration at 90th day (1.172 mg kg^{-1}) (CD=0.025; p=0.05). The statistical analysis showed that the Zinc concentration during the study time showed substantial increase in all the organs of the fish. The significant higher uptake was by gills (1.276 mg kg^{-1}) followed by the muscle (1.499 mg kg^{-1}), stomach (0.766 mg kg^{-1}) and brain (0.751 mg kg^{-1}) (CD=0.039; p=0.05). It was the heart of the fish that accumulated the least zinc metal (0.503 mg kg^{-1}).The interaction between Zn concentration in fish organs and duration was also significant during the present study (CD=0.055; p=0.05) (Table 4). It is known that zinc (Zn) deficiency is connected to hypogonadism, loss of

Fig. 1 : Sewage treated pond

Fig. 2: Fish organs for heavy metal estimation

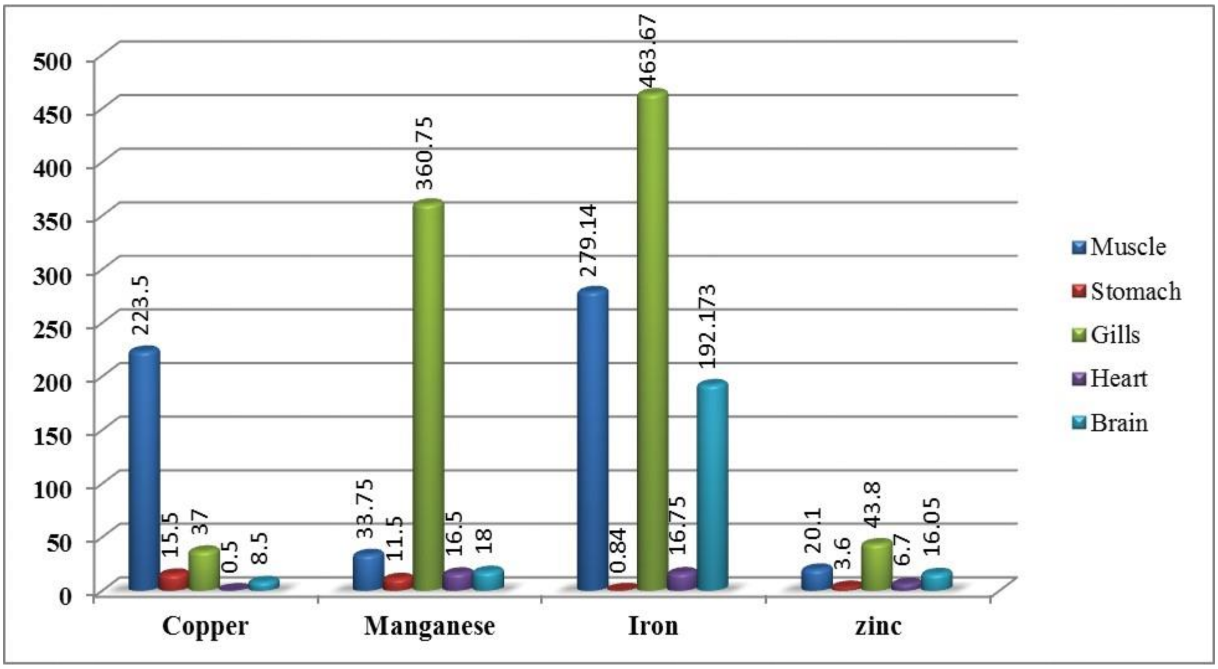

Fig. 3: Net bioaccumulation of heavy metal (bioaccumulation factor) in fish cultured in sewage treated water

taste, and retarded growth (WHO 2011). However, high levels of zinc and zinc-containing compounds are hazardous, humans are more likely to experience adverse effects on their digestive, haematological, respiratory and cardiovascular systems, as well as their neurological and cardiovascular systems (U.S. Department of Health and Human Services, 2003). According to FAO guidelines, the maximum concentration of Zn is 30 µg/g. Based on the present fish samples, the Zn levels were within FAO guidelines. The results of the present study was also corroborated by Akan *et al*. (2012) where the measurement of Zn concentrations in the gill of *T. zilli* (3.45 µg g^{-1}) was found to highest and the flesh of *C. angullaris* (0.06 µg g^{-1}) have the lowest content.

Bioaccumulation of heavy metals in Pangasius hypopthalamus

Bioaccumulation of heavy metals in *P. hypopthalamus* cultured in sewage treated water is presented in Table 5 with the help of bio-accumulation factor as mentioned above. Copper, manganese, iron and zinc bioaccumulation was 186.5, 104.75, 104.16 and 64.9 in fish muscle at 0 day and 410, 138.5, 383.3 and 85 at 90 days respectively. In gills, copper, manganese, iron and zinc bioaccumulation was 55.5, 241.75, 120.83, 41.9 at 0 day and 92.5, 602.5, 584.5, 85.7 at 90 day respectively (Table 5). In the tissue of *P. hypopthalamus* such as heart, brain and stomach, the bioaccumulation of heavy metals ranged from 21.8 to 107, 21.5 to 228.33 and 36.5 to 99.5 respectively. Metal bioaccumulation patterns in fish are largely determined by their affinity for metals and on the rate of their uptake, deposition, and excretion (Jezierska and Witeska, 2006). Under routine metal contamination monitoring, muscle is the major tissue of interest since humans consume it. The muscle tissue of *P. hyophthalmus* accumulated Fe (9.2 mg kg^{-1}) maximally, succeeded by Cu (3.280 mg kg^{-1}), Zn (1.70 mg kg^{-1}) and Mn (1.108 mg kg^{-1}) at 90^{th} day.

Kumar and Li (2018) pointed out high levels of lead and copper in the gills and liver of two fish, *Pelteobagrus fluvidrac* and *C. carpio* in Meiliang Bay of Lake Taihu. In the muscle tissue of *T. niloticus* collected from Lake Manzala, Shafei (2015) observed high toxicity of copper. The lake's south part may have a high copper load due to the constantly discharged sewage treatment. Similarly, Eneji *et al.* (2011) recorded the bioaccumulation factor for copper to be 178 and 105 in the muscle of *T. zilli* and *C. gariepinus*, respectively captured from river Benue (Nigeria). Additionally, Kumar *et al.* (2011) found that muscle tissue from *L. rohita* procured from Eastern Kolkata Wetlands contained high mean Zinc concentrations (0.7 to 5.0 mg g^{-1}).

Net Bioaccumulation of heavy metal in fish cultured in sewage treated water

According to the results estimated, Fe > Mn > Cu > Zn represents the net accumulation of heavy metals. (Figure 3). Organ wise, gills had maximum bioaccumulation of manganese (360.75), iron (463.67) and zinc (43.8). Muscle tissue of the fish had more progressive accumulation of copper (223.5) than other heavy metals. The net accumulation of iron, manganese, copper and zinc in L kg^{-1} in the heart tissue was 16.75, 16.5, 0.5 and 6.7. In case of tissue of stomach, the net accumulation was 0.84, 11.5, 15.5 and 3.6, and for brain tissue, 192.17, 18, 8.5 and 16.05 respectively. Among the five fish organs, gills had the maximum bioaccumulation whereas stomach had the minimum bioaccumulation of heavy metals. Among the five fish organs, Gills > Muscle > Brain > Heart > Stomach represented the order of bioaccumulation.

During the current investigation, the stomach tissue of *P. hypophthalmus* absorbed Iron maximally (1.802 mg kg^{-1}) surpassing Zn (0.802 mg kg^{-1}), Mn (0.796mg kg^{-1}) and Cu (0.208 mg kg^{-1}). Akan *et al.* (2012) showed that in comparison to other species, the stomach of *Synodentis budgetti* accumulated significantly more Fe, owever the stomach of *T. zilli* accumulated the most Zn. Gills are the main respiratory organs of the fishes and the maximum bioaccumulation site also. *P. hypophthalmus* gills accumulated mostly Fe metal (14.02 mg kg^{-1}) followed by Mn (4.820 mg kg^{-1}), Zn (1.714 mg kg^{-1}) and Cu (0.370 mg kg^{-1}) which was corroborated by earlier works. Heart being the main circulatory organ absorbed Iron (1.356 mg kg^{-1}) maximally succeeded by Mn (0.856 mg kg^{-1}), Zn (0.570 mg kg^{-1}) and Cu (0.130 mg kg^{-1}). The concentration of heavy elements in heart tissue, according to Ashraf (2005), was Zn > Cu > Pb > Co > Ni > Mn > Cd. Possibly, Zn's higher accumulation rate the (34.53 ± 9.96 ppm) in the fish tissues is due to the fact that zinc is a bio essential element, so by the process of homeostasis its concentration is maintained within a specific range (Falconer *et al.*, 1983). The concentration of Copper was found minimum in the heart tissue of *P. hypophthalmus.* A similar pattern of metal accumulation, Zn >Cu >Pb>Co >Ni >Mn>Cd was observed in the heart tissue. The observations recorded for the brain of *P. hypophthalmus* showed that the maximum absorption of heavy metal was by iron (5.480 mg kg^{-1}) succeeded by Zn (1.072 mg kg^{-1}), Mn (0.894 mg kg^{-1}) and Cu (0.124 mg kg^{-1}).

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