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Original Scientific Paper

Trend analysis of heavy metal contamination and arsenic levels in complete feed for fish and other complete animal feeds

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ABSTRACT

The aim of this research was to analyse the levels of toxic heavy metals (Mercury, Lead and Cadmium) and a toxic metalloid (Arsenic) in fish feed and animal feed from Bosnia and Herzegovina for the period from 2013 to 2018. Data from the National Veterinary Inspection Sector were provided for this study (n=438). The mean levels of As, Hg, Pb and Cd in all fish feed samples were 0.90±0.50 mg/kg, 0.02±0.05 mg/kg, 0.42±0.70 mg/kg and 0.16±0.11 mg/kg, respectively. During 2013–2018, the mean levels of As, Hg, Pb and Cd in animal feed (other complete feed) were 0.42±1.22 mg/kg, 0.01±0.02 mg/kg, 0.75±2.18 mg/kg and 0.10±0.12 mg/kg, respectively. In animal feeds sampled between 2013–2018, mean annual Pb levels increased the most among the four elements studied. In contrast, mean annual As, Hg and Cd levels in animal feeds continuously decreased during the study period. The mean annual Pb level in fish feed decreased, but continuous increases were observed in mean annual Hg and Cd levels during the study. The results show that the levels of toxic elements in fish feed and animal feed require attention and deserve a high priority monitoring program, as most feeds complied with the regulated maximum allowed concentrations of As, Hg, Pb and Cd in Bosnia and Herzegovina and in the European Union, but some did not.

1. Introduction

Agricultural and industrial development has been responsible for much heavy metal contamination of soils and waters. The major routes of heavy metal input to agricultural soils include animal manures, fertilizer and livestock products (*Nicholson et al.,* 1999). Hazards from the group of industrial-chemical pollutants include heavy metals and organic chemical contaminants. Due to the protection of consumer health, a number of heavy metals in foods is limited by the regulations in most European countries. This primarily refers to mercury, lead, cadmium, arsenic, and in some cases to other heavy metals such as zinc, tin, copper and iron. Animal feed and feed materials can be contaminated with heavy metals. This is very important, because these elements, after ingestion by animals, can then transfer along the food chain to be present in foods of animal origin, causing them to be a risk for human health (*Adamse et. al*., 2017).

Pb, Hg, Cd and As are the most toxic elements for animal health (*Bampidis et al.,* 2013; *Suttle*, 2010). Heavy metal toxicity can occur in animals from high ambient air concentrations near emission sources, or by eating contaminated feed (*Pandey and Madhuri*, 2014; *Castro-Gonzalez et al*., 2008). These

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metals are toxic since they are bioaccumulated in animal liver and kidney with potent effects of mutagenicity, carcinogenicity, teratogenicity and immunosuppression. Pb is a toxic, bioaccumulative heavy metal that has no known biological function. It accumulates in high concentrations in bones, teeth, liver, lungs, kidneys, brain and spleen, and it poses a serious risk to the health of the human population (*Castro-Gonzalez et al*., 2008). The most pronounced toxic effects of Pb are expressed on the nerve, haematological, cardiovascular and kidney systems (*Agency for Toxic Substance and Disease Registry*, 2005). Cd is not essential to any organism, and it is also not known to have any biological function in mammals. Hg is a cumulative poison, so symptoms of poisoning depend on the frequency or quantity of the input (*National Research Council (NRC)*, 2005; *Lopez-Alonso*, 2012; *Mandal and Suzuki*, 2002). As accumulates in the liver, from which it slowly releases and distributes into the stomach, intestines, the nervous system, and skin, and it tends to be deposited in bones and skin and permanently in the teeth (*Gwalteney-Brant et al.,* 2002).

Within the European Union (EU), the maximum allowable concentrations (MAC) of As, Pb, Hg and Cd in animal feed are regulated in Directive 2002/32/EC, last amended by Regulation 2015/186 (*Commission Regulation (EU) 2015*). Along with the EU regulation, national monitoring of toxic element concentrations in animal feed and fish feed is very important. It is necessary to have insight into the contamination and levels of toxic elements in the feed for different animal species. However, there are no data about heavy metal levels in animal feed or fish feed in Bosnia and Herzegovina. Accordingly, the aim of this study was to analyse the trends of heavy metal contamination and As levels in fish feed and animal feed between 2013 and 2018 and compare the levels found with literature data.

2. Materials and Methods

2.1 Sampling

Data from the National Veterinary Inspection Sector were provided for this study. Data covered the monitoring results of four toxic elements (As, Pb, Hg and Cd) in fish feed and animal feed in Bosnia and Herzegovina between 2013 and 2018. Animal feed that is submitted for heavy metal testing is partly sampled by the border veterinary inspection, and partly originates from internal traffic, in accordance with the prescribed competences for the purpose of controlling the production and circulation of animal feed in the country and ensuring the appropriate control and health correctness of animal feed. The number of samples (n=438) of fish feed and complete animal feed examined annually between 2013 and 2018 is presented in Table 1.

2.2 Toxic element analysis

All chemicals used were analytical grade purity. Digestion of feed was performed using a microwave closed system, MW 3000 (Anton Paar GmbH, Graz, Austria). Digestion was carried out with programs suitable for preparing samples of feed. After digestion, the content of toxic elements (As, Pb, Cd) in the feeds was determined by atomic absorption spectrometry using a Perkin Elmer Analyst 700 with the MHS system (Shelton, USA). Quality of analyses was controlled using certified reference material. The concentrations determined in the reference material were within the tolerances specified in the delivered certificate.

The amount of Hg was determined by direct burning on the Hg analyser, AMA-254. The principle of Hg determination is based on the quantification of Hg from homogenized samples that are weighed in a

Table 1. Number of fish and complete animal feeds examined in the study

Year	Fish feed	Animal feed	Total
2013	3	–	3
2014	58	25	83
2015	73	3	76
2016	68	16	84
2017	34	42	76
2018	32	84	116
Total			**438**

container. The sample thus prepared is transferred to the incineration furnace where it is dried and decomposed in a stream of oxygen at 850 °C. The decomposed products pass through a catalytic furnace at 700 °C where nitrogen oxides and sulphur are retained. Hg is captured on the amalgamator. The amalgamator is heated for a short period, and the Hg vapour released is transported to the measuring cells. Hg atoms absorb the radiation emitted by the Hg lamp, and based on the absorption of light at the appropriate wavelength, the concentration of Hg in the sample is determined. In proportion to the increase in the number of atoms, the amount of light absorbed by the atoms also increases, and by measuring the amount of absorbed light, it is possible to determine the amount of Hg in the sample.

The levels of heavy metals and As were compared with the MAC in animal feed established by the EU (*Commission Regulation (EU)*, 2015) and by Bosnia and Herzegovina (*Official Gazette of Bosnia and Herzegovina*, no. 72/11, 70/16).

2.3 Statistical analysis

All samples were collected and analysed in triplicate, and the results were expressed as mean±standard deviation. Analysis was elaborated using GraphPad Prism version 7.00 software. The coefficient of determination (R^2) was used to evaluate the significance of potential trends of the elements' levels in the feeds between 2013 and 2018 (Microsoft Office, Excel, 2010). The trends with R^2 values exceeding 0.30 were considered significant (*Adamse et al.,* 2017).

3. Results and discussion

The mean heavy metal and As levels in the fish feed samples analysed between 2013 and 2018 are presented in Table 2. In general, the heavy metals detected in fish feed samples did not exceed the MAC regulated in Directive 2002/32/EC, last amended by Regulation 2015/186 (*Commission Regulation (EU)*, 2015) and the Official Gazette of Bosnia and Herzegovina (no. 72/11, 70/16). However, mean Hg levels in some fish feeds were above the MAC of 0.1 mg/kg (Table 2). The mean annual levels of As, Hg, Pb and Cd in all fish feed samples were 0.90±0.50 mg/kg, 0.02±0.05 mg/kg, 0.42±0.70 mg/kg and 0.16±0.11mg/kg, respectively. The elements detected at the highest levels in the fish feeds from Bosnia and Herzegovina were As and Pb, which ranged from 0.18–2.98 mg/kg and 0.03–4.30 mg/kg, respectively.

The As, Hg, Pb and Cd concentration in complete animal feed samples were in the range of 0.10–6.39 mg/kg, 0.01–0.09 mg/kg, 0.10–13.69 mg/kg and 0.02–0.54 mg/kg, respectively. According to the EU (*Commission Regulation (EU)*, 2015) and the *Official Gazette of Bosnia and Herzegovina* (no. 72/11, 70/16), the MAC of Pb in complete animal feed is 5 mg/kg, so some animal feed samples in the present

Table 2. The heavy metal and arsenic levels in fish feeds (mg/kg) (2013–2018)

Parameter	Elements			
	As	Hg	Pb	Cd
Mean±SD	0.90±0.50	0.02±0.05	0.42±0.70	0.16±0.11
Median	0.71	0.01	0.20	0.12
Range	0.18–2.98	0.01–0.52	0.10–4.30	0.04–0.65

Legend: According to the European Union (Commission Regulation (EU), 2015) and the Official Gazette of Bosnia and Herzegovina (no. 72/11, 70/16), the MAC of As, Hg, Pb and Cd in fish feed is 10 mg/kg, 0.2 mg/kg, 5 mg/kg and 1 mg/kg, respectively.

Table 3. The heavy metal and arsenic levels in complete animal feeds (mg/kg) (2013–2018)

Parameter	Elements			
	As	Hg	Pb	Cd
Mean±SD	0.42±1.22	0.01±0.02	0.75±2.18	0.10±0.12
Median	0.06	0.002	0.25	0.06
Range	0.01–6.39	0.01–0.09	0.10–13.69	0.02–0.54

Legend: According to the European Union (Commission Regulation (EU), 2015/186) and the Official Gazette of Bosnia and Herzegovina (no. 72/11, 70/16), the MAC of As, Hg, Pb and Cd in complete animal feed is 2 mg/kg, 0.1 mg/kg, 5 mg/kg and 1 mg/kg, respectively.

study did not conform with the prescribed MAC (Table 3). As in fish feeds, Pb was the toxic element detected at the highest levels in complete animal feeds (Table 3).

According to *EFSA* (2015), As in animal feed originates from geological sources, industrial activities and specific feed additives. Also, drinking water can be one of the inorganics As sources (*Mandal*, 2002; *Lopez- Alonso*, 2012; *Bampidis at al*., 2013). As contents in the fish feed varied greatly in the examined years (Figure 1). As was not detected in fish feed samples in 2013 or 2014. The mean As level in fish feed was significantly (R^2=0.885) higher in 2015, 2016 and 2017 than in 2018. The mean annual As level in fish feed followed the order 2013<2014<2018<2017<2016<2015. According to the *Commission Regulation (EU)* (2015) and the *Official Gazette of Bosnia and Herzegovina* (no. 72/11, 70/16), the MAC of As in complete animal feed is 2 mg/kg, so some complete animal feed samples in the present study did not conform with the prescribed MAC. *Adamse et al*. (2017) showed that seaweed accumulates As. Similar results were presented in a study by *Makkar et al*. (2016). *Wang* (2013) showed that feed additives for animal production can have a high risk of unacceptable As levels. According to a study by EFSA (*European Food Safety Authority*, 2015), high levels of As were found in fishmeal. Total As concentrations in complete feeds were shown by the EFSA contaminants panel (*European Food Safety Authority*, 2015), when the mean As level in complete feeds for beef cattle, broilers and pigs (grower/finishers) was 0.36 mg/kg, 0.34 mg/kg and 0.31 mg/kg, respectively. In the current study, As levels between 2013 and 2018 were higher in fish feed than in complete animal feed. Mean annual As levels did not vary significantly in complete animal feeds from 2013 to 2018 (Figure 1). As was once used for disease control in farm animals, but now, in Europe, its use is forbidden in animal production (*Li et al.*, 2005).

Trend analysis of Pb levels in complete animal and fish feeds between 2013 and 2018 is shown in Figure 2. The highest mean annual Pb level was found in complete animal feed from 2017. Each year of the study, the mean Pb level was below 2 mg/kg. In the fish feed samples, mean Pb levels decreased during 2013–2018. However, Pb levels in complete animal feed increased in that period. The reason for this upward trend of Pb levels is unexplained. Pb is a very toxic element and is an indicator of environmental pollution caused by anthropogenic factors (*Sager*, 2007). As a toxic element, Pb levels in mineral supplements and premix for animals can be higher than in other livestock feedstuffs. The EFSA contaminants panel (2004a) stated Pb levels in complete feeds for beef, poultry, broilers and pigs were 1.14 mg/kg, 1.16 mg/kg, 0.52 mg/kg and 1.03 mg/kg, respectively. In the study by *Wang*

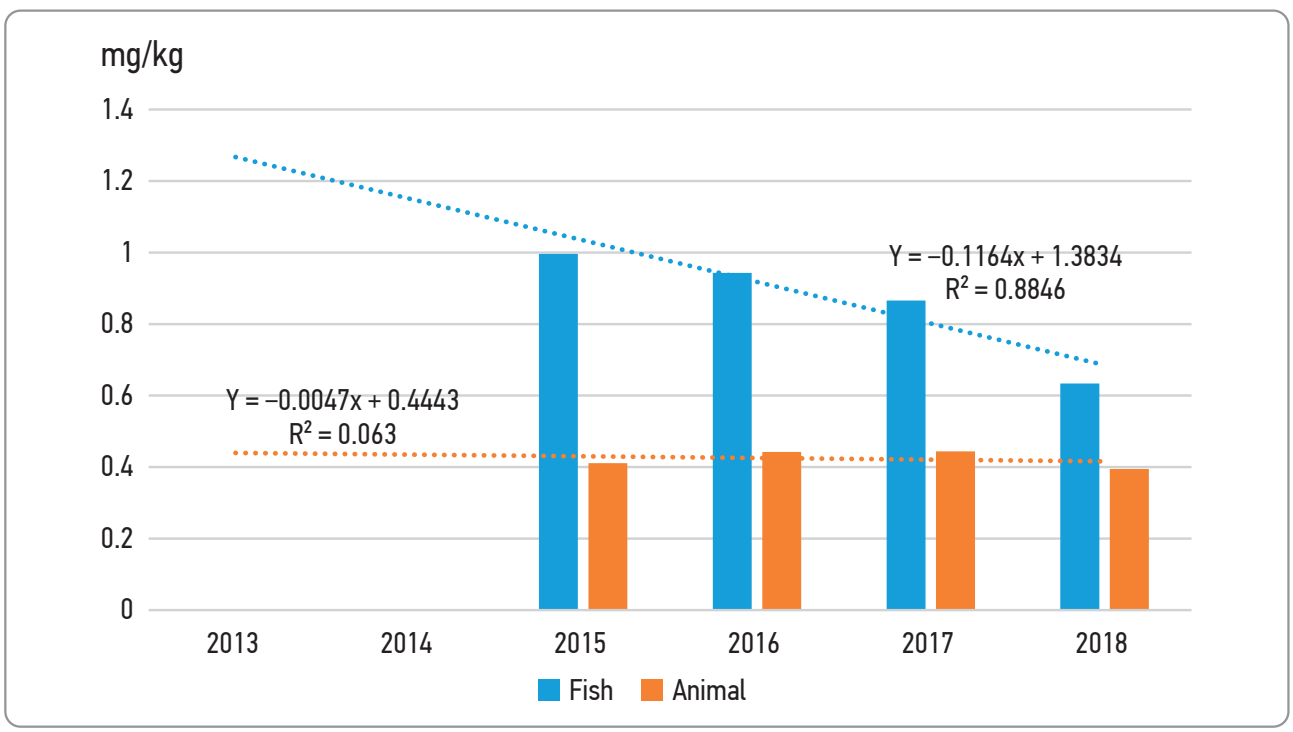

Figure 1. The mean levels of Arsenic in complete animal and fish feeds, between 2013 and 2018.

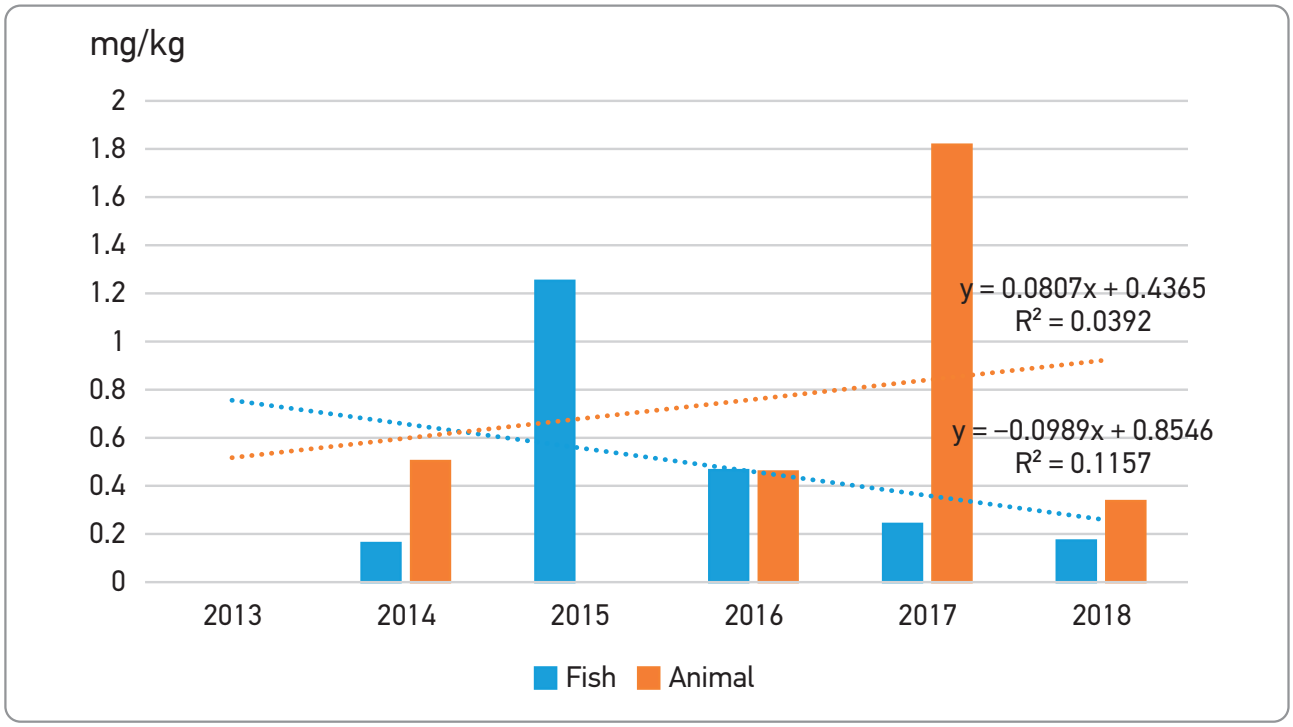

Figure 2. The mean levels of Lead in complete animal and fish feeds, between 2013 and 2018.

et al. (2013), a high correlation between Pb levels in the environment and in animal manure was found. During 2013–2018, Pb levels increased in the complete animal feed in our study, which could be due to the increased use of mineral supplements in animal nutrition. The monitoring program for Pb contamination in the environment showed that Pb levels decreased in all European countries (*European Food Safety Authority*, 2015).

Significantly decreasing mean annual levels of Hg were found in complete animal feed samples (R^2=0.60) for all the tested period, 2013–2017

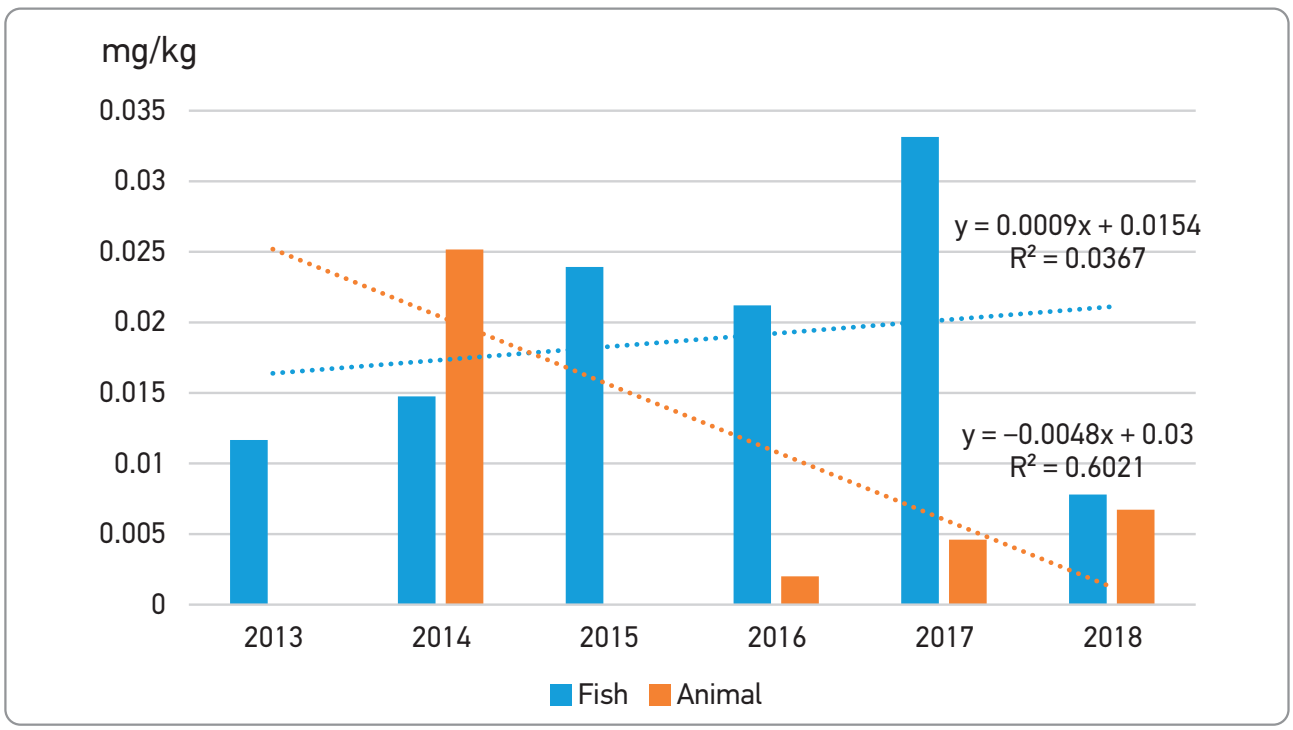

Figure 3. The mean levels of Mercury in complete animal and fish feeds, between 2013 and 2018.

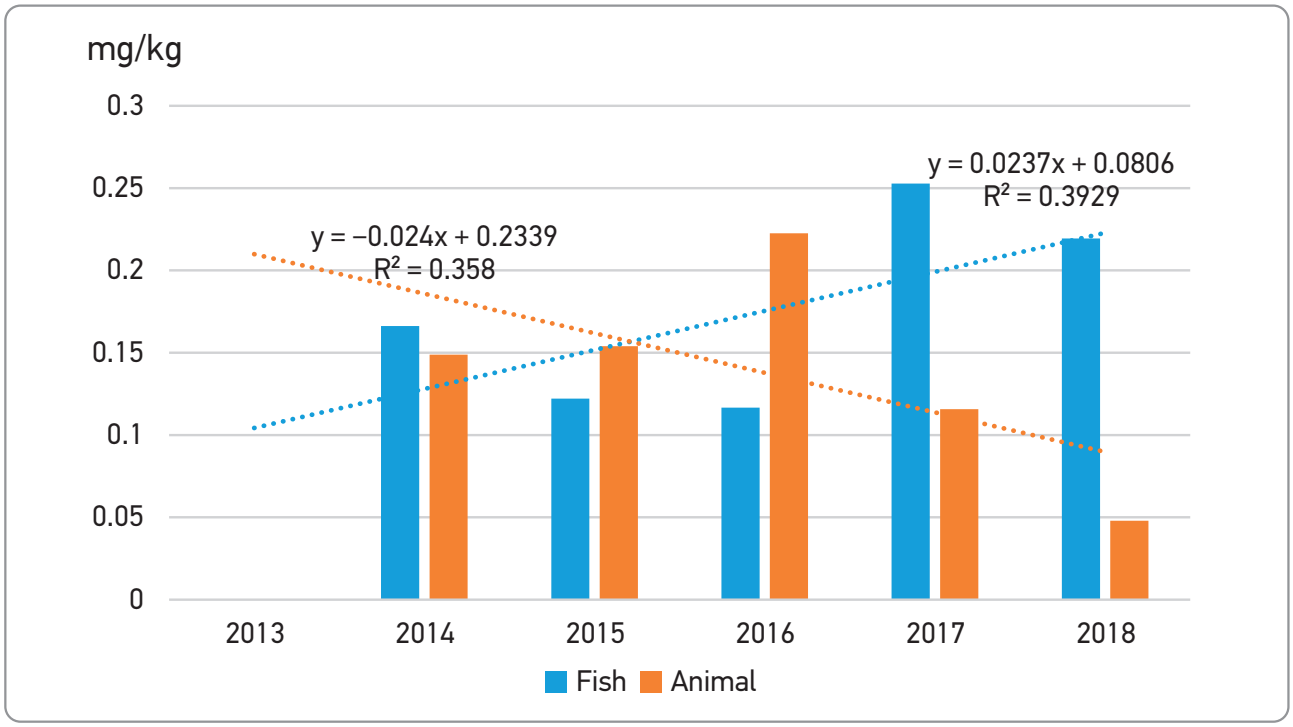

Figure 4. The mean levels of Cadmium in complete animal and fish feeds, between 2013 and 2018.

(Figure 3). However, mean annual levels of Hg in fish feed increased during this period. Compared with the other examined years, in complete animal feed samples during 2017, a higher mean Hg level was found. Similarly, to Pb, Hg is a toxic element and indicator of environment pollution (*Nicholson et al*., 2017; *Li et al.*, 2005). Fish feed and other fish meals are the major sources of Hg in animal nutritional products utilized in livestock production (*Lopez- Alonso*, 2012). The Hg concentrations in feed supplements and additives are generally low. According to EFSA (*European Food Safety Authority*, 2008), higher Hg concentrations were found in rodent complete feeds (0.050 mg/kg), poultry complete feeds (0.039 mg/kg) and pig complete feeds (0.032 mg/kg). In the present study, mean annual Hg levels in both fish and complete animal feeds were below the MAC (0.2 mg/kg and 0.1 mg/kg, respectively) established by the EU (*Commission Regulation (EU)*, 2015) and the *Official Gazette of Bosnia and Herzegovina* (no. 72/11, 70/16). In a previous study (*Adamse et al*., 2017), Hg levels in animal feed decreased from 2007–2013.

The mean annual Cd levels in fish feed and animal feed between 2013 and 2018 are presented in Figure 4. Significantly decreasing mean Cd levels were found in complete animal feed samples (R^2=0.35) during these years. However, mean Cd levels significantly increased in fish feed during 2013–2018 (R^2=0.39). Mean annual Cd levels in the fish and complete animal feeds were below the MAC (1.0 mg/kg) established by the EU (*Commission Regulation (EU) 2015/186*) and the *Official Gazette of Bosnia and Herzegovina* (no. 72/11, 70/16). High Cd levels in fish feed could be due to agricultural activities (use of fertilizers) (*Lopez-Alonso*, 2012, *Rajaganapathy et al.*, 2011, *Amlund et al*., 2012). Also, according to *McBride* (1998) and *Dai et al*. (2016), mineral supplements can contain high Cd levels. The ESFA contaminants panel (2004b) stated Cd levels in complete feeds for poultry, broilers, ruminants and pigs were 0.16 mg/kg, 0.19 mg/kg, 0.11 mg/kg and 0.09 mg/kg, respectively. *Adamse et al*. (2017) showed that Cd levels significantly increased between 2007 and 2013 in feed material of marine origin. One of the most sensitive animal species for Cd toxicity is the pig (*King et al*., 1992).

4. Conclusion

It is necessary to have insight into the contamination and levels of toxic elements in the feed for different animal species. However, there are no data about heavy metal levels in animal feed or fish feed in Bosnia and Herzegovina. Accordingly, the aim of this study was to analyse the trends of heavy metal contamination and As levels in fish feed and animal

feed between 2013 and 2018 and compare the levels found with literature data. The results show that the levels of toxic elements in fish feed and animal feed require attention and deserve a high priority monitoring program, as most feeds complied with the regulated maximum allowed concentrations of As, Hg, Pb and Cd in Bosnia and Herzegovina and in the European Union, but some did not.

Analiza trenda kontaminacije teškim metalima i nivoa arsena u kompletnoj hrani za ribe i drugih potpunih smeša za ishranu životinja

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INFORMACIJE O RADU

Ključne reči:
Hrana za ribe
Hrana za životinje
Teški metali
Nivo
Toksičnost.

APSTRAKT

Cilj ovog istraživanja bio je utvrditi nivo toksičnih metala (olovo, kadmijum, živa i arsen) u hrani za ribe i stočnoj hrani iz Bosne i Hercegovine za period od 2013. do 2018. godine. Podaci Sektora nacionalne veterinarske inspekcije su korišćeni za ovu studiju (n=438). Srednji nivoi As, Hg, Pb i Cd u svim uzorcima hrane za ribe bili su 0,90±0,50 mg/kg, 0,02±0,05 mg/kg, 0,42±0,70 mg/kg i 0,16±0,11 mg/kg, pojedinačno. Tokom 2013–2018, srednji nivoi As, Hg, Pb i Cd u stočnoj hrani (ostala potpuna hrana za životinje) bili su 0,42±1,22 mg/kg, 0,01±0,02 mg/kg, 0,75±2,18 mg/kg i 0,10±0,12 mg. /kg, pojedinačno.. U hrani za životinje uzorkovanoj između 2013–2018, srednji nivoi Pb su se najviše povećali. Nasuprot tome, srednji nivoi As, Hg i Cd u stočnoj hrani kontinuirano su opadali tokom perioda istraživanja. Srednji nivo Pb u hrani za ribe se smanjio, ali su uočeni kontinuirani porasti srednjih nivoa Hg i Cd tokom studije. Rezultati pokazuju da nivoi toksičnih elemenata u hrani za ribe i stočnu hranu zahtevaju pažnju i zaslužuju program praćenja visokog prioriteta, iako As, Pb i Hg nisu prekoračili maksimalno dozvoljene koncentracije regulisane Direktivom 2002/32/EC, poslednjom izmenjenom Uredbom. 2015/186 i Službeni glasnik Bosne i Hercegovine (72/11, 70/16).

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