Bioaccumulation of inorganic elements in *Dreissena polymorpha* from the Ebro River, Spain. Could zebra mussels be used as a bioindicator of the impact of human activities?

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Running Head: Inorganic elements accumulation in zebra mussels and human activity.

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Abstract

Dreissena polymorpha is among the top one hundred most harmful invasive species in aquatic habitats. European Directive 2013/39/UE establishes Environmental Quality Standards (EQS) for biota because it has been demonstrated that pollutants bioaccumulate in aquatic organisms. This study evaluated bioaccumulation of inorganic elements in the soft tissues of *Dreissena polymorpha* in order to assess the usefulness of zebra mussels as a bioindicator of contaminant presence in superficial waters. Concentrations of 66 elements were measured in order to evaluate their relationship with nearby anthropogenic activity and to the values recommended by EQS for biota. Bivalves were collected from four sample points along the Ebro River Basin (Spain), where diverse human activities are carried out. Zebra mussels accumulate toxins in soft tissue during their life cycle, including Al, Cr, Fe, Hg, Pb, Th, Cd, and U. The highest levels of accumulation corresponded to elements associated with human activity in the area, showing the impact of anthropogenic actions on biota. *Dreissena polymorpha* not only supplies information about current water quality but acts as a witness of past water quality by bioconcentrating toxic elements present in the environment and providing relevant results about historical water contamination. In conclusion, *Dreissena polymorpha* is a harmful and dangerous invasive species, but its pervasiveness means that it can be used as a bioindicator to assess current and past presence of elements in water.

Introduction

The spread of invasive species is considered a serious ecological problem and is one of the major causes of biodiversity loss worldwide (European commission, 2013). The guidelines by the International Union for Conservation of Nature (IUCN) intend to prevent biodiversity loss caused by (invasive / non-native) species and stress the need to identify the most harmful invaders (ISSG, 2000; Katsanevakis et al., 2013; Matthews et al., 2015). *Dreissena polymorpha*, a freshwater bivalve commonly known as the zebra mussel, is among the top 100 most harmful invasive species according to the IUCN (Lowe et al., 2000), and its great reproductive and colonization capacity makes it possible for it to quickly adapt to new aquatic habitats.

The Ebro River is one of the largest and most abundant rivers in Spain with a basin area of 85.362 km^2 that goes through northeastern Spain and discharges into the Mediterranean Sea. Numerous reservoirs are found along the length of the Ebro, and its affluent rivers, which serve to provide water for consumption purposes and for the production of hydroelectricity. The river supplies water to a population of more than three million people. –The banks of the river are surrounded by areas with human residences, industrial zones, and farms which is a cause for concern because the water runoff may contribute to contamination of the river (Terrado et al., 2006). In Spain, invasion of the Ebro River basin by *Dreissena polymorpha* was described for the first time in 2001 (Ruiz-Altaba et al., 2001). The largest populations of zebra mussels have been detected in reservoirs, since the most suitable habitats for *D. polymorpha* are stable riverbeds (Durán et al., 2007) with flow rates below 1.2 $m\ s^{-1}$, and a depth of less than 5 m under regular flows (Sanz-Ronda et al., 2014). Because these bivalves are filter feeders they can bio-accumulate and concentrate high amounts of microbiological and chemical pollutants in their soft tissue (Magni et al., 2015). An adult individual of *Dreissena polymorpha* is able to filter between 1 and 2.5 liters of water per day (Claudi and Mackie, 1994; CHE, 2007), and is capable of processing water at rates of 600 ml per hour (Costa et al., 2011; Elliott, 2005). The bivalve's capacity to accumulate both chemical and microbiological pollutants has been documented in numerous research studies. This makes the zebra mussel a useful tool for bio-monitoring freshwater ecosystems where they can be found attached to the substrate (Alcaraz et al., 2011; Bourgeault et al., 2011; Camusso et al. 2001; Mosteo et al., 2016, Palos Ladeiro et al., 2014; Voets et al., 2009).

European legislation has introduced environmental quality standards (EQS) for biota, through EU Directive 2013/39/UE, which includes 45 priority substances that require EQS, and 8 labeled as "certain other pollutants." Some very hydrophobic substances are hardly detectable in water even using the most advanced analytical techniques but accumulate in biota, where EQS should be set . The directive states that in order for member states to carry out monitoring successfully by adapting it to their local circumstances they should have sufficient flexibility in the application of the EQS or, where relevant, the ability to utilize an alternative biota taxon, such as class Bivalvia, as a bioindicator. The implementation of the EU Directive has increased the demand for information about bioindicator species and their applicability for monitoring pollutants. Currently the EQS in the area of water policy are regulated in Spain by the RD 817/2015 which also establishes acceptable concentration levels for sixteen preferential substances in water because they are considered to be contaminants that present a significant risk in Spanish surface waters due to their toxicity, persistence, and bioaccumulation.

The present work studies the accumulation of 66 elements in zebra mussels collected from four different points along the Ebro River in order to evaluate how they compare with the established EQS for biota. Each collection site has a different combination of industrial, livestock, and agricultural activities carried out in the vicinity. In addition, the acquired results were compared with elemental concentrations in the water itself, from the preceding three years, obtained by the network that monitors the status of the surface water in the Ebro River basin, in order to investigate the possible usefulness of bivalves as bioindicator of past anthropogenic pollution.

Materials and Methods

Study area

Zebra mussel specimens were collected from four different sampling points along the Ebro River basin: three of them from reservoirs and the fourth from an irrigation channel. The sampling sites are as follows (Fig. 1.A-B):

- Point 1: The Sotonera Reservoir is situated downstream of the passage of the Gállego River through Sabiñánigo (Huesca, Spain) and collects freshwater from the affluent rivers Gállego (around 86% of the reservoir volume), Astón and Sotón. The reservoir water is used to generate hydroelectricity and for irrigation. The Sotonera reservoir is downstream from an industrial cluster, where many of the industries are or were involved in the manufacture of chemical products such as: sulfuric acid, electrolytic aluminium, chlorine, HCH, phosphorous, and others, thereby generating waste that lingers in the environment(i.e.: Lindane) (Fernández et al., 2013; Val et. al, 2016) (Fig. 1.C).

- Point 2: The Flix reservoir, located in a lower basin meander, has an area of 320 km^2, a volume of 11 hm^3, and a water residence time of 0.15 days, thus it maintains some of the properties of a river (Carrasco et al., 2011). The reservoir is used for hydro electrical generation and regulation. A notable feature of this reservoir is a chlor-alkali plant, located on the right bank, that has caused the deposition of industrial wastes in the adjacent riverbed (approximately 35×10^4 t) (Alcaraz et al., 2011; Boixadera et al., 2015; Bosch et al., 2009; Carrasco et al., 2008, 2011). For decades, this deposit has accumulated industrial wastes from the plant with high levels of organochlorine compounds, trace metals, metalloids, and radionuclides (Alcaraz et al., 2011). The concentration of trace metals in the layer of sediment in contact with the industrial sludge is estimated to be at 18-170 mg kg^{-1} Hg, 0.66-8.4 mg kg^{-1} Cd, 16-22 mg kg^{-1} As, 20-34 mg kg^{-1} Se, 72-290 mg kg^{-1} Zn, 34-96 mg kg^{-1} Cu, 150-490 mg kg^{-1} Cr, and 16-52 mg kg^{-1} Pb (Soto et al., 2011). Moreover, storage depot deficiencies have caused filtrations of several pollutants including Al, Ba, Cd, Cr, Cu, Fe, Ni, Pb, and Zn into the soil and surface water, and this information has been noted and published by local newspaper (Fig. 1.D).

- Point 3: The Sobrón reservoir (Burgos, and Álava, Spain) is located in the upper course of the Ebro River and is used to produce hydroelectricity. Livestock farming and agricultural activities take place in the area around the reservoir. (Fig. 1.E).

- Point 4: The Rimer irrigation channel (Caspe, Spain) is adjacent to the Guadalope River near to its confluence with the Ebro River in the Mequinenza reservoir. This is also an agricultural area (Fig. 1.F).

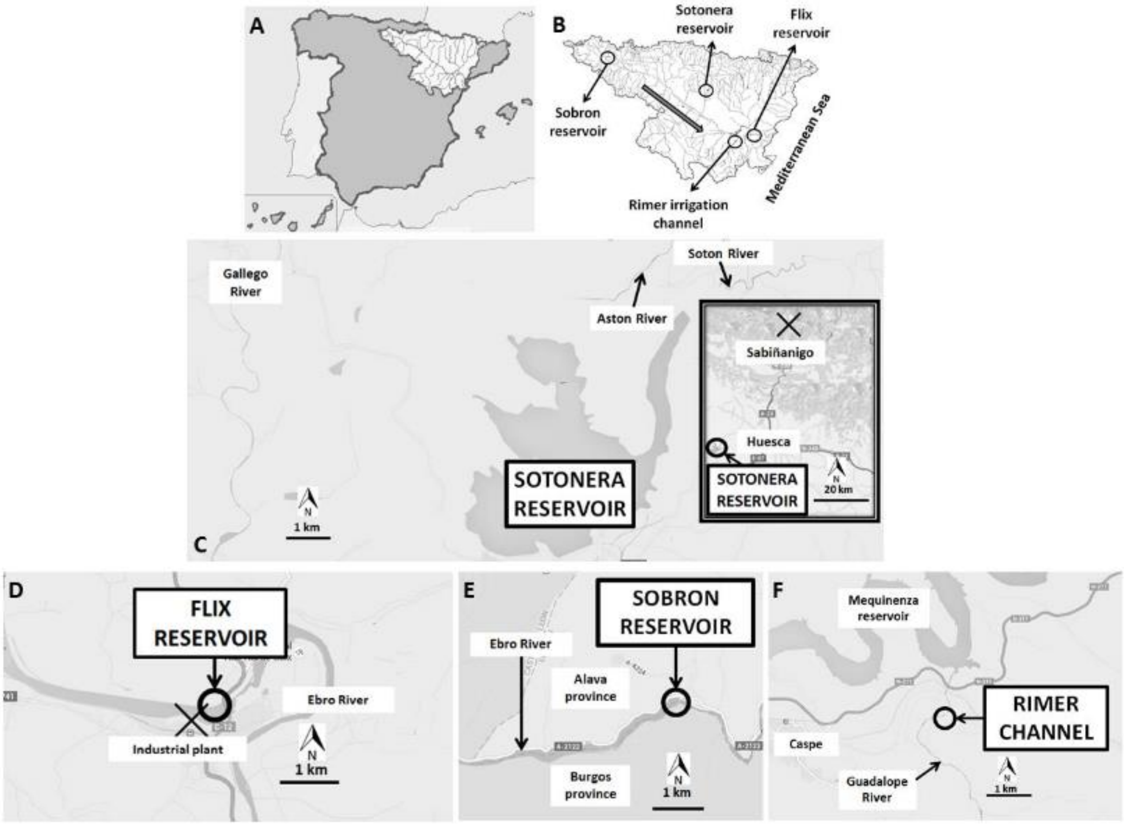

Figure 1. A: Ebro River basin in Spain. B: Sampling sites (www.chebro) C: The Sotonera Reservoir. D: The Flix Reservoir. E: The Sobron Reservoir. F: Rimer irrigation channel

Sample collection

Zebra mussel specimens were hand collected from rocks, and sediments in the reservoirs or irrigation channel, between March 20th and June 3rd 2014, placed in a sterile container with a wide mouth, and maintained at a temperature of approximately 20^{0}C in an isothermal container during the transfer to the laboratory. The specimens selected for the study, were adult individuals with a valve size between 1.5-2 centimeters. Fifty mussels were collected at each sample point, from which around 10 individuals with similar valve sizes were selected randomly in order to acquire a heterogeneous sample. The protocol for disinfecting the equipment used during the sampling process, was validated by the Hydrographic Confederation (CHE 2002; Anadón et al., 2012). The selected specimens were processed immediately after the sampling.

Analytical methods

For metal analysis, the zebra mussel specimens were rinsed at least five times with Milli-Q water. Soft tissue was then dissected from the valve and byssus, and stored at -20ºC (Gundacker, 1999).

Once the samples were collected, 1.1125 [0.972-1.294] grams of soft tissue were freeze-dried in a Lioalfa 6-50 lyophilizator at -40°C with 10^{-1} bar vacuum pressure (Jović and Stankovic., 2014) during 24 hours. The product obtained in each sample point was pooled. Two hundred milligrams from each sample were taken, 7 mL of HNO_3 (J.T. Baker CAS NO 7697-37-2) and 3 mL of HCl were added (J.T. Baker 7647-01-0) and the tissue was digested at 200^0C during 30 minutes in a CEM MARS-X model microwave. Afterwards, up to 50 mL of Milli-Q water was added and the metal concentration was semi-quantitatively determined by Inductively Coupled Plasma-Mass Spectrometer analysis (ICP-MS). Three different measurements were performed for each sample point.

Positive and negative controls and certified standard materials (Trace elements certified material TMDA-70.2, and SLRS-5, Environment Canada and National Research Council of Canada respectively Canada, Resource Technology Corporation US, CRM 052) were used for quality control and quality assurance. Errors in a semi-quantitative determination by ICP-MS can reach 30%.

The concentrations of 66 elements were studied (Table 1) and the minimum determinable concentration (MDC) of each metal was estimated as ten times the standard deviation of three zero adjusted readouts.

Data analysis

Since there are no universally accepted reference levels for elements accumulated in *Dreissena polymorpha*, in order to assess what concentrations would be indicative of water pollution, a base level was established as the lowest value found in tissue.Taking into account the error of the method, it was defined that a value that exceeds more than three times the lowest found in all of the sample points was an indicator of pollution.

Four of the analyzed metals, Cd, Pb, Hg and Ni, appear in the EU Directive 2013/39/UE as priority substances and certain other pollutants establishing EQS in water. Of them, only Hg has established values for EQS in biota, which allowed for a direct comparison with the results of this study. Five other elements figure in RD 817/2015 as preferential substances (As, Cu, Cr, Se, and Zn) and have values set for EQS in water.

The data obtained from the mussels were compared to the concentrations of the analyzed elements present in the water, provided by the website of The Ebro River Hydrographic Confederation (CHE) (http://www.datossuperficiales.chebro.es:81/WCASF/?rvn=1), selecting for each sample point the nearest monitoring station located in a ratio down to 25 km.

Results and Discussion

Metal bioaccumulation in zebra mussels

Concentrations of the 66 elements which were detected in the soft tissues of the collected mussels are shown in Table 1. It should be noted that 36 of the analyzed elements did not reach the MDC in bivalves from any of the sampling points: 14 of them in mussels from all points, other 6 in mussels from Sotonera reservoir, 7 in mussels from Sobron Reservoir and 9 in those from the Rimer irrigation Chanel. No additional elements were found in the mussels from Flix Reservoir at levels lower than the MDC.

A distinction must be made between elements essential to the mollusk's survival and metals without a metabolic function that are generally classified as toxic. Among the essential elements are Cu, Fe, Mn and Zn whose concentrations are regulated by the mussel through uptake and excretion mechanisms (Bervoets et al., 2004). The latter group includes metals such as Cd, Cr, Hg, or Pb whose bio-concentration depends largely on the environment. They tend to be eliminated very slowly, and are not often regulated by excretion or uptake control systems (Voets et al., 2009).

No special mention is made of Na, Mg, Al, Si, P, K, Ca and Fe because their high concentrations are related to the properties of the surrounding soil, although some of them appear in higher concentrations than three times the lowest result obtained in any sample point.

Table 2 summarizes the most significant results obtained in this work for the elements whose concentration in mussel soft tissue is considered significant according to the criteria established in section "*Data analysis*". Values that were more than three times the minimum value obtained, but had a concentration lower than 1 µg g^{-1}, are not included in Table 2, specifically Y, Zr, Nb, Pr, Sb, La, Nd, Sm, Gd, Dy, Er and Tl, so it was considered that these results represent minor amounts of metal to evaluate them as determinant results.

Data obtained in a literature review for polluted and not polluted areas, to compare with the values obtained in this study, are compiled in table 3.

Table 4 shows the maximum and minimum concentration of select elements in water, during the three years preceding this study, of the sites where zebra mussels were sampled, the data was provided by the CHE. All of the measured concentration levels during the three years comply with the average yearly EQS set by the Directive 2013/39/ UE and RD 817/2015 (AA-EQS).

The highest concentrations of Cr, Ni, Cu, W and Au were found in mussels from the Sotonera Reservoir and this is also the only site where the Au concentration in samples exceeded the MDC. The chromium concentration in mussels from this reservoir (67.26 µg g^{-1}) was significantly higher than the measurements obtained at the other sampling points. In the case of Al, considerably higher amounts appeared in specimens from the Sotonera and Flix reservoirs (833.07 µg g^{-1} and 1447.86 µg g^{-1} respectively) compared with those from Sobrón or Rimer (100.19 µg g^{-1} and 151.98 µg g^{-1} respectively).

The zebra mussel specimens from the Flix Reservoir accumulated the highest concentrations of the elements analyzed, especially those related to industrial activity Elements that appeared in higher concentrations in the sludge deposited on the right bank of the reservoir were also found to have higher concentrations in the soft tissue of the mussels.

The highest concentrations of Ni were detected in mussels from the reservoirs located at the most industrialized areas: The Sotonera (18.64 µg g^{-1}) and Flix (8.7 µg g^{-1}) reservoirs. When the results were compared with concentrations from other studies (Table 3) it was noted that both concentrations found in mussels from the Sobrón (5.85 µg g^{-1}) and Rimer (7.16 µg g^{-1}) were within non polluted range.

Similar levels for Cu and Zn were observed among mollusks from all of the sampled sites. The Cu concentrations found in *D. polymorpha* tissue were between 7.17 µg g^{-1} and 13.37 µg g^{-1}. In other studies (Table 3), the range for bivalves captured in non-polluted waters was between 2.42- 26.3 µg g^{-1} and in polluted waters between 10.20-35.75 µg g^{-1} although there is a significant overlap between these ranges. The Zn concentration ranged from 97.49 µg g^{-1} in mussels from the Sotonera Reservoir to 117.73 µg g^{-1} in those from Rimer irrigation channel. These results coincide with those from other studies which found 99.4-246.19 µg g^{-1} of Zn in mussels sampled in non-polluted areas and 94.4-346 µg g^{-1} of Zn in those found in polluted areas. In this case there is also an overlap between the polluted and no-polluted areas. Copper is an essential hemocyanin constituent in mollusks and Zn is involved in the formation and function of several enzymes. Therefore, both metals are essential oligo-elements for the mussel which means their internal concentrations need to be regulated for survival and likely explains the fact that their values have no correlation with those found in the environment (Gundacker, 1999). Internal concentrations of Mn can also be regulated by the mussels, but they can be affected by external conditions (Rzymski et al., 2014).

The highest level of Cd appeared in bivalves from the Sobrón Reservoir (2.51 µg g^{-1}) followed by those from the Rimer irrigation channel (1.28 µg g^{-1}) which are both surrounded by areas with agricultural and livestock activities. The lowest values were found in mussels from the Sotonera and Flix reservoirs (0.62 µg g^{-1} and 0.64 µg g^{-1} respectively) which are in areas with high industrial activities. Arsenic (As) was also found in higher concentrations in mussels from the Rimer and Sobrón reservoirs, with levels of 7.50 µg g^{-1} and 6.59 µg g^{-1} respectively. The concentrations of As in mussels from the Sotonera and Flix reservoirs were lower, 3.91 µg g^{-1} and 4.13 µg g^{-1} respectively. A similar pattern was observed for other metals: B, Na, Ca, Sr, Hg, and Mo were found to be most highly concentrated in zebra mussels from Sobrón Reservoir and the highest concentrations for Si, U, and Zn were found in mussels from the Rimer irrigation channel. When this data is compared with that of other studies (Table 3), Cd levels found in zebra mussels from the Sobrón and Rimer are within the same range as mussels inhabiting polluted waters (1.51-3.96 µg g^{-1}) while levels in mussels from the Sotonera (0.62 µg g^{-1}) and Flix (0.64 µg g^{-1}) reservoirs are within the range found for those that inhabit non-polluted waters (0.23-0.78 µg g^{-1}). High concentrations of Cd and As may be attributed to the use of phosphate fertilizers, herbicides, fungicides, and insecticides (Hartley et al., 2013; Macedo et al.; 2009). Concentrations of As, may not only be influenced by anthropogenic activities, but also by lithological changes in the surrounding land (Terrado et al., 2006).

The presence of Pb, Hg, and Cr in the environment is almost entirely attributed to contamination caused by human activity. As was expected, the value for Pb concentration (1.78 µg g^{-1}) yielded by zebra mussels from Flix reservoir were within the range described for mollusks that inhabit contaminated areas (0.71-5.87 µg g^{-1}) because this element was a significant component of the sludge deposited in this reservoir (Soto et al., 2011). The lowest concentration of Pb was detected in mussels from the Sobron reservoir (0.19 µg g^{-1}) and intermediate concentrations were detected in samples from the Sotonera and Rimer (0.58 µg g^{-1} and 0.45 µg g^{-1} respectively) all of these values were below the range for mussels inhabiting polluted areas in previous studies.

Although the highest concentration of Hg was expected in mussels from the Flix reservoir, because of the presence of a chlor-alkali plant situated close to the reservoir which produces high amounts of industrial waste with high Hg concentrations of up to 436 mg g^{-1}(Carrasco et al., 2008), it was found that mussels from the Sobron reservoir had the highest levels of Hg, probably due to its presence in fertilizers (Fatta et al., 2007). The amounts of Hg found in the zebra mussels from Sotonera Reservoir and Rimer irrigation channel did not reach the minimum determinable concentration. The concentrations found in Flix and Sobrón were 0.10 µg g^{-1} and 0.15 µg g^{-1} respectively. Mercury is the only metal that has EQS for biota in the Directive 2013/39/UE. The EQS indicate that there should be no more than 20 µg of Hg per kg^{-1} of wet weight. Considering the water content in zebra mussels to be 90% it was calculated that the limit for Hg would be approximately 0.02 µg per g^{-1} of dry weight of zebra mussel. Thus the Hg detected in the mussels from both the Flix and Sobrón reservoirs comply with the EU Directive and its transposition to Spanish Regularion (Real Decreto 817/2015).

The chromium concentration detected in zebra mussels from the Sotonera Reservoir is notable because there was a high level of Cr detected in the soft tissue of the mussels (67.26 µg g^{-1}) compared with the other sample locations where the concentrations were within the range of 2.68-4.65 µg g^{-1}. It is also considerably higher than the maximum level (4.97 µg g^{-1}) reported in the literature that was reviewed, which was found in Lake Maggiore, Italy (Camusso et al., 2001). These levels can only be explained by the presence of anthropogenic pollution which is consistent with the fact that the sample site is located near an area where industrial activities are carried out.

Previous research on the digestive glands of *Mytilus galloprovincialis*, a brackish-water mussel, reported a concentration of iron of 524 µg g^{-1} in mussels from a non-polluted area (Forte dei Marmi, Italy) and 782 µg g^{-1} in those found at a polluted site (Scarlino, Italy) (Betti et al., 2003). The Fe levels detected in the Flix and Sotonera mussels (1287.81 µg g^{-1} and 952.19, respectively) are higher than the concentration found in Scarlino, which suggests that these zones are significantly affected by industrial pollution. In contrast, the Sobrón and Rimer mussels concentrations were even lower than those found in the non-polluted area in the *Mytilus galloprovincialis* study.

The results obtained for the Ba concentration in zebra mussels from the Flix (17.48 µg g^{-1}) and Sotonera (12.29 µg g^{-1}) reservoirs were higher than three times the lowest value detected in those captured in Rimer (3.91 µg g^{-1}). The highest concentrations of this metal were detected in areas with a proximity to industrial activities, likely because Ba is often found in industrial waste sludge.

There is no information in the literature regarding Al, Cs, Th, and Sb concentrations in *Dreissena polymorpha*. Comparing the results obtained from the bivalves from each sample site the highest concentrations for these four elements appear in mussels from the Flix Reservoir, followed by those from the Sotonera where concentration levels of all these metals excepting Sb that do not reach the MDC. The Al concentration detected in mussels from Flix was especially high (1447.86 µg g^{-1}), more than fifteen times greater than the lowest value found for Al in this study (10.19 µg g^{-1} in mussels from Sobrón). Other studies concerning the uranium presence in this area have found the highest values for uranium activity in the most superficial samples taken from the sludge sediments near the dicalcium phosphate factory, especially in the first meter of sediment depth (Mola et al, 2013). However, a higher level of uranium in the soft tissue of mussels was detected in the Rimer irrigation channel. The Rimer irrigation channel is located close the Mequinenza Reservoir where there is a lignite field that has significant S, Mo and U enrichment, the U content being estimated at 26 ppm. This quantity represents twelve times the European average for this element in carbon (Querol et al., 1996; Álvarez, 1995). This may explain why the highest concentration appears precisely in Rimer mussels. There are no results concerning uranium in the data provided by the water monitoring stations (Table 4) or in the literature (Table 3).

Metal concentrations in surface water

In order to examine the possible existence of a direct relationship between the studied element concentrations in the soft tissue of zebra mussels and in the surrounding water, the network that monitors the status of the surface water in the Ebro basin which is conducted by CHE provided the concentration of these elements in the water. Maximum and minimum concentration levels for each element, during a 3 year period before sample collection (2012, 2013 and 2014), are shown in Table 4. The *Dreissena polymorpha* lifespan is approximately 3 years (Durán et al., 2007) during which time the mussel filters water in the ecosystem that it occupies.

The data shows that the levels of Ni, Cu, and Zn in superficial waters were low (minimum of detection or close to it) during the three relevant years. However, levels of these metals in the zebra mussels indicate their accumulation in the bivalve tissue.

The results for concentration of As in water had values within the range of 0.00027-0.0020 mg L^{-1}. The highest concentration was found in the Rimer irrigation channel where the zebra mussels also had the highest concentrations of this semimetal in their soft tissue. The results of the Cd concentrations in mussels and water again show a correlation between the highest results in soft tissue and water. The highest value for Cd among the maximum concentration data compiled in all the locations appears in the Sobrón reservoir (0.00009 mg L^{-1}) corresponding with the sample sites with highest values in mussels.

Unlike As whose origin may be related to lithological changes in the soil, other metals such as Pb, Hg and Cr are related to human activity and contamination. The Pb concentration in surface water captured by the monitoring stations in the Sotonera reservoir, Flix reservoir, and the Rimer irrigation channel during the three years was ≤0.0005 mg L^{-1} while the Sobrón reservoir had a range of <0.0005-0.0018 mg L^{-1}. Therefore, there is no correlation between water concentrations of Pb and accumulation in *Dreissena polymorpha* since the highest result in the sampled bivalves was detected in the Flix reservoir (1.78 µg g^{-1}) and the lowest in the Sobrón reservoir (0.19 µg g^{-1}).

The level of Hg in the surface water collected by the monitoring stations was <0.000012 mg L^{-1} in all the sites, suggesting that the bivalves capture Hg and bioaccumulate it in their soft tissue during their life cycle, although it is not perceptible in water.

The Cr levels in water measured by the CHE monitoring stations were lower than 0.0020 mg L^{-1} in all the locations during the three years before the mussel harvest. In a control station situated near the industrial center of Sabiñánigo, the Cr concentration in water was often monitored and ranges from 0.0020 to 0.0322 mg L^{-1}. This means that the concentration limit, for the environmental quality standards (EQS) applicable to surface water, established in RD 817/2015 (EU Directive 2013/39/UE) is respected (annual mean value lower than 0.005 mg L^{-1} for Cr VI and 0.050 mg L^{-1} for Cr). This demonstrates that Cr is accumulated in zebra mussels during their life cycle, because the concentration in their soft tissue is much higher than in the surface water in the ecosystem where they are found. Other studies have demonstrated that despite the slow uptake of Cr by the zebra mussel, *Dreissena polymorpha* is a suitable organism for monitoring this metal (Soto et al., 2011).

The information acquired about the Fe concentration in water shows the lowest value in Flix, while there are no records for this metal in the Sobrón reservoir biomonitoring station. There is consequently no correspondence between the water and soft tissue results.

There was a correlation between the concentrations of Ba in water and the bivalve levels at all of the sample points. The Flix had the highest values both for the maximum and minimum concentrations, followed by the Sotonera reservoir and then the Rimer concentrations (for both maximum and minimum levels). No results have been recorded by monitoring stations for the Sobrón reservoir.

There were no concentration differences for Sb between the locations. There is no information about this element in the Sobron Reservoir. Information about the Al, Cs, Th, and U concentrations in water is also unavailable.

Elements are mainly accumulated in sediments, bio-accumulated in aquatic biota, as is verified by the present study, and bio-magnified along the food chain. In addition, some elements may have harmful effects on the environment, wildlife, fauna, agricultural products and human health, even in low concentrations (Alcaraz et al., 2011). *Dreissena polymorpha* specimens bio-accumulate metals and other substances during their whole life cycle. These elements become concentrated inside the mussels while remaining more diluted in the waterbody. Consequently they are sometimes present in significantly lower levels in water than in mussels (Table 4).

It has been shown in this study that reservoirs with industrial water intake, such as the Flix and Sotonera, have considerably higher concentrations of those elements associated with industrial production than agricultural areas in zebra mussels. However, in agricultural areas concentration peaks of elements usually found in fertilizers and other agricultural products are frequently detected. It would be of interest to establish associations between the concentrations of contaminants in water and in *Dreissena polymorpha* by monitoring and sampling the bivalves repeatedly during at least three years in order to establish the possible usefulness of zebra mussels as an indicator for detecting possible discharges.

Conclusion

This study reveals that some elements, some of them heavy metals that are found in water bodies, are bioaccumulated and concentrated in the soft tissues of *Dreissena polymorpha*. These sometimes toxic elements are diluted in the superficial water in which the mussels are found, but they are retained inside of the bivalves, in a quantity that depends on the concentration of the elements in the environment, due to their slow or inexistent removal from the tissues. A correlation was observed between high concentrations of some elements that can act as indicators of anthropogenic activity in *Dreissena polymorpha* and concentrations in superficial water, which shows the impact of these activities on biota and the environment. In consequence, the zebra mussels not only provide information about the current situation of inorganic elements in surface waters but also supply historical data about the area where the specimens live, acting as witnesses by bio-concentrating toxic metals.

This situation poses a serious risk for ecosystems due to the redistribution of the pollutants, and also for public health in two ways first by the liberation of metals from inside the mussels when the specimens die and also by the possible transfer to upper trophic levels.

Conflict of Interest

We have not conflicto of interest

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Table 1: Metal concentrations (µg g^{-1} dry weight) in *Dreissena polymorpha* soft tissue at various sample points, and the Minimum Determinable Concentration (MDC) of each metal.

Metal	Sotonera reservoir	Flix reservoir	Sobrón reservoir	Rimer i. channel	MDC	Metal	Sotonera reservoir	Flix reservoir	Sobrón reservoir	Rimer i. channel	MDC
Li	1.45	2.54	0.27	0.42	0.02	**Sn**	<MDC	<MDC	<MDC	<MDC	0.1
Be	0.06	0.08	<MDC	<MDC	0.04	**Sb**	<MDC	0.17	0.03	0.04	0.01
B	<MDC	0.92	1.10	<MDC	0.61	**Te**	<MDC	<MDC	<MDC	<MDC	0.06
Na	466.25	762.07	1675.53	1322.57	22.74	**Cs**	0.24	0.35	0.05	0.055	0.01
Mg	664.57	955.01	798.80	933.60	0.33	**Ba**	12.29	17.48	5.53	3.91	0.05
Al	833.07	1447.86	100.19	151.98	8.76	**La**	0.43	0.90	0.17	0.19	0.01
Si	205.35	64.63	190.30	287.64	4.69	**Ce**	<MDC	1.42	<MDC	<MDC	0.36
P	5738.01	7062.68	6330.52	6646.46	17.08	**Pr**	0.11	0.21	0.04	0.06	0.01
K	831.08	2278.90	1404.72	1286.42	3.98	**Nd**	0.37	0.85	0.15	0.165	0.01
Ca	7225.84	10604.49	14801.30	8819.35	4.76	**Sm**	0.08	0.18	0.03	0.04	0.01
Sc	3.63	3.70	1.44	1.91	0.78	**Eu**	0.02	0.04	<MDC	<MDC	0.01
Ti	28.77	45.71	24.94	27.71	0.91	**Gd**	0.08	0.18	0.02	0.04	0.01
V	<MDC	<MDC	<MDC	<MDC	4.56	**Tb**	0.01	0.02	<MDC	<MDC	0.01
Cr	67.26	4.65	3.38	2.68	0.07	**Dy**	0.05	0.11	0.02	0.03	0.01
Mn	40.19	72.89	16.43	41.39	0.09	**Ho**	<MDC	0.02	<MDC	<MDC	0.01
Fe	952.19	1287.81	353.08	461.34	34.24	**Er**	0.03	0.05	0.01	0.01	0.01
Co	0.90	1.13	0.89	0.92	0.02	**Tm**	<MDC	<MDC	<MDC	<MDC	0.01
Ni	18.64	8.70	5.85	7.16	0.48	**Yb**	0.02	0.04	<MDC	<MDC	0.01
Cu	13.37	13.06	8.09	7.17	0.37	**Lu**	<MDC	<MDC	<MDC	<MDC	0.01
Zn	97.49	110.50	98.38	117.73	1.04	**Hf**	0.01	0.01	<MDC	<MDC	0.01
Ga	0.41	0.67	0.10	0.11	0.01	**Ta**	<MDC	<MDC	<MDC	<MDC	0.01
As	3.91	4.13	6.59	7.50	1.21	**W**	0.38	0.24	0.18	0.22	0.09
Se	<MDC	<MDC	<MDC	<MDC	3.25	**Re**	<MDC	<MDC	<MDC	<MDC	0.01
Rb	3.18	6.40	2.46	2.40	0.02	**Os**	<MDC	<MDC	<MDC	<MDC	0.01
Sr	66.95	62.73	86.01	60.75	0.06	**Ir**	<MDC	<MDC	<MDC	<MDC	0.01
Y	0.24	0.51	0.08	0.13	0.01	**Pt**	<MDC	<MDC	<MDC	<MDC	0.17
Zr	0.21	0.51	0.09	0.24	0.06	**Au**	0.03	<MDC	<MDC	<MDC	0.01
Nb	0.09	0.10	0.02	0.04	0.01	**Hg**	<MDC	0.10	0.15	<MDC	0.08
Mo	0.13	0.34	0.36	0.15	0.04	**Tl**	0.03	0.05	0.01	0.015	0.01
Ru	<MDC	<MDC	<MDC	<MDC	0.01	**Pb**	0.58	1.78	0.19	0.45	0.06
Pd	<MDC	<MDC	<MDC	<MDC	0.03	**Bi**	<MDC	<MDC	<MDC	<MDC	0.05
Ag	<MDC	0.68	<MDC	<MDC	0.24	**Th**	0.21	0.35	0.04	0.06	0.01
Cd	0.62	0.64	2.51	1.28	0.03	**U**	0.08	0.21	0.15	0.31	0.01

Table 2: Summary of elements with notable concentration results in *Dreissena polymorpha* soft tissue (µg g^{-1} dry weight).

	Elements regulated by European Directive 2013/39/UE and RD 817/2015									Elements with other noted results available								
Origin	**Cr**	**Se**	**Ni**	**Cu**	**Zn**	**As**	**Cd**	**Hg**	**Pb**	**Al**	**Mn**	**Fe**	**Sb**	**Ba**	**Cs**	**Th**	**Mo**	**U**
Sotonera	67.26	<MDC	18.64	13.37	97.49	3.91	0.62	<MDC	0.58	833.07	40.19	952.19	<MDC	12.29	0.24	0.21	0.13	0.08
Flix	4.65	<MDC	8.70	13.06	110.50	4.13	0.64	0.10	1.78	1447.86	72.89	1287.81	0.17	17.48	0.35	0.35	0.34	0.21
Sobrón	3.38	<MDC	5.85	8.09	98.38	6.59	2.51	0.15	0.19	100.19	16.43	353.08	0.03	5.53	0.05	0.04	0.36	0.15
Rimer	2.68	<MDC	7.16	7.17	117.73	7.50	1.28	<MDC	0.45	151.98	41.39	461.34	0.04	3.91	0.06	0.06	0.15	0.31
MDC	0,07	3.25	0.48	0.37	1.04	1.21	0.03	0.08	0.06	8.76	0.09	34.24	0.01	0.05	0.01	0.01	0.04	0.01

Table 3: Metal concentrations in *Dreissena polymorpha* (μg g^{-1} dry weight) found in other research projects.

	Location	Cr	Ni	Cu	Zn	As	Cd	Hg	Pb	Mn	References
Non polluted areas	Garda lake, Italy	2.87±0.28	12.1±2.4	18.1±3.6	158±27		0.78±0.43	0.065±0.006	1.96±0.48		Camusso et al., 2001
	Lugano lake, Italy	2.03±0.19	11.9±1.7	26.3±8.5	163±13		0.6±0,15	0.049±0.002	2.46±0.72		Camusso et al., 2001
	Oneida lake, USA	1.55	4.2	4.6	99.4		0.68	0.05	1.03		Secor et al., 1993
	Mequinenza reservoir, Spain	2.52±0.14	7.01±0.07	8.37±0.08	78.98±0.98	5.77±0.11	0.30±0.03	0.04±0.01	0.86±0.01	48.32±0.48	Alcaraz et al., 2011
	Marnay-surSeine, France	1.03	4.39	2.42	246.19		0.23				Bourgeault et al., 2011
	Riba-roja reservoir, Spain	0.62±0.15	9.77±1.68	7.91±1.18	92.50±9.56	6.19±0.12	0.31±0.03	0.10±0.03	0.50±0.15		Faria et al., 2009
	Groβ Enzersorfer Arm, Austria			7.6±0.4	111±15		0.71±0.30		0.22±0.08		Gundacker, 1999
Polluted areas	Como lake, Italy	4.55±0.41	24.2±1.7	14.6±3.0	247±10		2.06±0.93	0.053±0.006	3.08±0.96		Camusso et al., 2001
	Maggiore lake, Italy	4.97±0.88	18.2±1.4	25.2±1.0	346±22		3.44±0.23	0.158±0.007	5.87±0.71		Camusso et al., 2001
	Onondaga lake, USA	2.45	7.4	10.2	94.4		1.71	0.2	1.24		Secor et al., 1993
	Triel-sur-Seine, France	7.2	7.23	18.4	246.19		1.51				Bourgeault et al., 2011
	Flix reservoir (downstream), Spain	3.35±0.034	14.94±0.24	21.13±2.27	100.44±199	4.38±0.14	2.34±0.03	0.56±0.01	1.40±0.13	518.31±53.94	Alcaraz et al., 2011
	Flix reservoir (downstream), Spain	1.3±0.05	37.77±7.12	30.52±2.38	136.38±13.43	5.35±0.03	3.96±0.13	0.87±0.01	0.77±0.09		Faria et al., 2009
	Flix reservoir (dumping area), Spain	0.99±0.01	25.34±2.49	35.75±0.90	129.83±5.29	4.84±0.08	1.82±0.42	3.01±0.1	0.71±0.001		Faria et al., 2009

Table 4: Minimum and maximum water concentrations (mg L^{-1}) of metals during the three relevant years that correspond to the life expectancy of sampled *Dreissena polymorpha*, were provided by nearest monitoring station of the network for monitoring the status of the surface water in the Ebro river basin. The data at each sample point is provided and the corresponding results detected in the soft tissue of the zebra mussels in the present study (µg g^{-1} dry weight) is included in parenthesis for comparison.

	European Directive 2013/39/UE and RD 817/2015									**Other noted results**			
	Cr	**Se**	**Ni**	**Cu**	**Zn**	**As**	**Cd**	**Hg**	**Pb**	**Mn**	**Fe**	**Sb**	**Ba**
Sotonera reservoir	<0.0020	≤0.00020	<0.0020-0.0019	<0.0020-0.0021	<0.010	0.0005-0.00137	<0.000020-0.00003	<0.000012	≤0.0005	0.0026-0.0390	0.021-0,19	<0.0005	0.0298-0.0364
	(67.26)	(<MD)	(18.64)	(13.37)	(97.49)	(3.91)	(0.62)	(<MDC)	(0.58)	(40.19)	(952.19)	(<MDC)	(12.29)
Flix reservoir	<0.0020	0.00047-0.00060	<0.0020-0.0048	<0.0020-0.0027	<0.010	0.00125-0.00211	<0.000020-0.00007	<0.000012	<0.0005	0.0032-0.0344	0.009-0.069	<0.0005	0.0335-0.0418
	(4.65)	(<MDC)	(8.70)	(13.06)	(110.50)	(4.13)	(0.64)	(0.10)	(1.78)	(72.89)	(1287.81)	(0.17)	(17.48)
-Sobrón reservoir	<0.0020	<0,00020-0.00025	<0.0020-0.0039	<0.0020-0.0072	<0.010-0.017	0.00027-0.00112	<0.000020-0.00009	<0.000012	<0.0005-0.0018	-	-	-	-
	(3.38)	(<MDC)	(5.85)	(8.09)	(98.38)	(6.59)	(2.51)	(0.15)	(0.19)	(16.43)	(353.08)	(0.03)	(5.53)
Rimer irrigation channel	<0.0020	0.00047-0.00067	<0.0020-0.0038	<0.0020-0.0052	<0.010	0.00056-0.00220	<0.000020	<0.000012	<0.0005	0.0014-0.0082	0.010-0.072	<0.0005	0.0254-0.0302
	(2.68)	(<MDC)	(7.16)	(7.17)	(117.73)	(7.50)	(1.28)	(<MDC)	(0.45)	(41.39)	(461.34)	(0.04)	(3.91)