Available online at www.sciencedirect.com
SCIENCE DIRECT®
Aquaculture 254 (2006) 317 – 325
Aquaculture
www.elsevier.com/locate/aqua-online

Preliminary investigation on the enrichment of heavy metals in marine sediments originated from intensive aquaculture effluents

Carolina Mendiguchía, Carlos Moreno *, Manuel P. Mánuel-Vez, Manuel García-Vargas

Faculty of Marine and Environmental Sciences, Department of Analytical Chemistry, University of Cádiz, 11510 Puerto Real, Cádiz, Spain

Received 24 March 2005; received in revised form 26 October 2005; accepted 28 October 2005

Abstract

In the last decades, marine aquaculture has experienced an important development around the world. One of the main environmental impacts of these activities is the high amount of particulate matter discharged, which comes from uneaten food and faeces. Although the concentration of heavy metals in these particles is relatively low, the total amount of solids is so high that the accumulation of metals in the sediments may become an important problem. This fact has been mentioned in previous works, but it was not studied in depth. In this work, we demonstrate the accumulation of selected heavy metals (Cu, Zn, and Pb) and organic matter (estimated as Loss on Ignition, LOI) in marine sediments as a consequence of intensive marine aquaculture.

The study was carried out in an arm-of-the-sea of the Bay of Cádiz (SW Spain) where several intensive marine aquaculture facilities are located. The data obtained were statistically treated to determine whether the spatial and temporal gradients in heavy metals and organic matter in sediments were related to aquaculture. Although the area is relatively pristine in regards to heavy metals, aquaculture activities have increased metals (Zn, Cu, Pb) and organic matter concentrations significantly. The average enrichments were: 140%, 362%, 97% and 445%, respectively. The results suggested that trace metals' enrichment in the sediments may be attributed to the fish farm effluents, although metal concentrations are not likely to cause harmful effects in the marine ecosystem.

Keywords: Fish culture; *Sparus aurata*; Sediments; Copper; Zinc; Lead; Organic matter

1. Introduction

The number and diversity of contaminants in the marine environment has increased notably over the last years (Kennish, 1997; Luoma, 1990). One of the new activities affecting coastal environments is marine aquaculture. While this industry generates many economical benefits, it originates several adverse environmental effects (Tovar et al., 2000a,b). Among the most important negative effects of intensive aquaculture are those related with high loads of nutrients, suspended solids and organic matter (Uotila, 1991; Jambrina, 1999), giving cause to increased biological oxygen demand, excessive algae growth, etc. In addition, organic matter has an important effect on sediment composition (changing both physical and chemical characteristics) and acts as metal trap. Furthermore aquaculture activities could also modify the concentration of heavy metals in adjacent sediments by introducing high loadings of metals contained in the particulate matter present in the

* Corresponding author. Fax: +34 956 016460.
E-mail address: carlos.moreno@uca.es (C. Moreno).

0044-8486/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.aquaculture.2005.10.049

effluents, which may be up to several tons per day (Tovar et al., 2000b).

Heavy metals are accumulated in marine sediments, where they are incorporated in several biological and chemical cycles, affecting the water column and biota. On the other hand, chemical reactions can change the concentration of heavy metals in sediments and, as a consequence, in the overlying water (Luoma, 1990).

High concentrations of zinc and other metals in sediments have been reported in zones where intensive aquaculture in cages has been carried out (Schendel et al., 2004; Otero et al., 2005). In many cases the metal source has been related to the food used (Uotila, 1991; Chou et al., 2002; Belias et al., 2003). However, other authors (Urdaneta, 1995; Carbonell et al., 1998) have concluded that the influence of aquaculture in the concentrations of heavy metals in the sediments is practically negligible. Thus, it is clear that more exhaustive studies on this topic are required. In this work, the influence of aquaculture activities on the concentrations of different heavy metals (Cu, Zn and Pb) in the sediments was investigated.

1.1. Site description

This study was carried out in an arm-of-the-sea (AOTS) called San Pedro River. It was selected because of its strategic location in the Bay of Cádiz and because several aquaculture facilities are located along its banks (see Fig. 1). Located into the protected area of the Natural Park of the Bay of Cádiz, it was a tributary of Guadalete River, but it was artificially blocked 12 km from its mouth. Therefore, recent San Pedro River is a seawater inlet with the length of 12 km, width ranging between 45 and 60 m and maximum depth of 3–4 m. Its only water input is from tides from the Bay of Cádiz making water renewal in the upper part (aquaculture zone) to be very poor.

In a previous study, the existence of two different zones within the inlet was established according to water quality (Tovar et al., 2000a). The first one, with a length of about 8 km, is closer to the mouth. In this zone, there are no fish farms and a good water renewal from the Bay. As a consequence, this part is not impacted by aquaculture, and chemical composition of water was very similar to Bay water. In the second section, about 4 km long, three fish farms are located. One of them (A in Fig. 1) is devoted to the intensive culture of gilthead sea bream (*Sparus aurata*) and its effluents are considered the main source of materials received by the ecosystem. The second farm (B in Fig. 1) is devoted to mollusks production (*Crassostrea giga*), and the third facility (C in Fig. 1) is a public research institution on aquaculture. More information on farms' characteristics is given elsewhere (Tovar et al., 2000a,b). Previous results indicated that the water quality of this upper region has been impacted by the aquaculture activities (mainly by nutrients and suspended solids) and by limited water renewal from the Bay.

Another very important feature of this closed AOTS is that there are no other activities than marine aquaculture. Thus, the only inputs received by water are: aquaculture effluents, water renovation from the Bay and rainwater, and then any non-natural alteration of the sediments, must be related to the aquaculture activities. As a consequence, the site becomes an ideal natural laboratory to study the environmental effects of marine aquaculture, and then, to establish direct

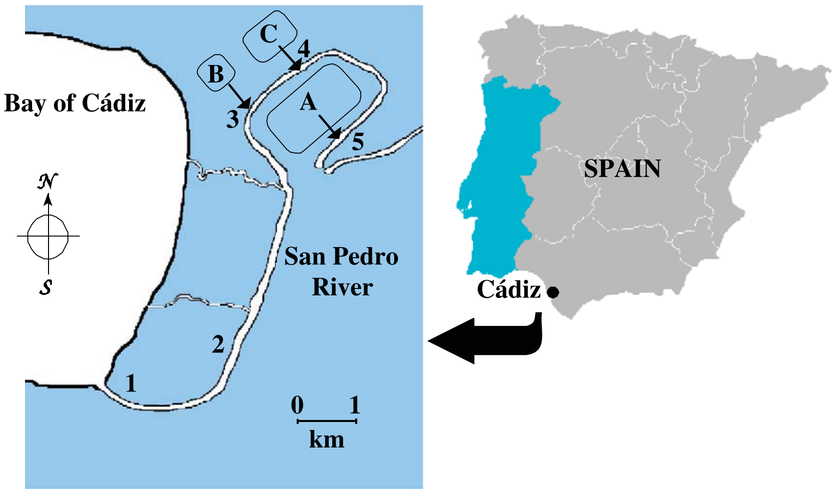

Fig. 1. Geographical situation of San Pedro River. A, B and C: aquaculture facilities (arrows indicate effluents); sampling sites denoted by numbers.

correlations between aquaculture and accumulation of heavy metals in sediments.

2. Materials and methods

2.1. Sampling

From the work on water quality mentioned above it follows that the study site may be characterized with five sampling stations. Sampling stations 1 and 2 were located in the first section of the AOTS (see Fig. 1), where the influence of fish farms effluents was negligible, and used as a pristine reference site. Sampling stations 3, 4, and 5 were located in the upper part, close to the effluents of B, C, and A facilities to quantify their impacts.

Two sampling campaigns were performed in February 1997 and March 1998. In each station, two samples of superficial sediments (about 900 cm^2 each) were collected with a Birge–Eckman type grab. The upper layer of each sample (2 cm) was separated, preserved in plastic containers and stored frozen until analysis.

To quantify direct production of heavy metals during a culture cycle, additional samples were collected in a selected aquaculture pond (in farm A). Before starting a new culture cycle, the bottom of the pond was deeply cleaned with a digger to remove solids from the last culture cycle. After that, we sampled three points of the pond (water input, middle and water output) and prepared a single composite sample, which was analyzed to estimate background metal concentrations in the pond. At the end of the two-year culture cycle, the pond was emptied and we took 6 samples of sediment: 2 in the zone of water input, 2 in the middle, and 2 in the output zone (all of them, equidistant to pond axis). Again, a composite sample was prepared and analyzed to estimate the concentrations of metals after a culture cycle.

Finally, two different fish feeds used in the farms were analyzed to determine their contribution to the accumulation of heavy metals (Zn, Cu, Pb) in sediments, via uneaten food and faeces.

2.2. Sample treatment and analysis

Prior to analysis, sediment samples were sieved through a 0.5-mm sieve, dried at 105 °C for 24 h, and then ground to fine powder with an agate mortar and stored in plastic vials (Jackson, 1982). The results of this work are expressed as the mean obtained from the analysis of the two samples collected in each station.

To dissolve the samples, approximately 0.2 g were, after calcination, digested twice with hydrofluoric acid (40%), and then, twice with nitric acid (65%). After drying, samples were extracted with hydrochloric acid (37%) and heated for 1 h. The solution obtained was filtered with an Albet 240 filter, and diluted to 25 mL. Finally, an Atomic Absorption Spectroscopy (AAS) instrument with flame atomizer (SolaarM, Thermo, UK), was used to determine the concentrations of Zn, Cu and Pb in the samples. The analytical procedure was verified by the analysis of Zn, Cu and Pb in a reference material (Marine Sediment GBW 07313, National Research Centre for Certified Reference Material, China). This material was analyzed in duplicate, and the accuracy of the results obtained was confirmed by applying the *t*-test at a 95% confidence level.

The organic matter of the sediments was evaluated as Loss on Ignition (LOI), by calculating the weight difference before and after ignition at 500 °C during 3 h. This is considered the method of estimation of organic matter most significant for the chemistry of trace elements in sediments (Horowitz and Elrick, 1987).

Food samples were ashed and digested by heating with nitric acid and hydrochloric acid for 1 h. After digestion, the solution was filtered with an Albet 240 filter, and diluted to 25 mL. Metal concentrations were determined by AAS.

2.3. Data treatment

Parametric two-way analysis of variance (ANOVA) was applied to the results to establish the significance, with a probability of 95%, of spatial and temporal variations in metal concentrations in the sediments. When significant variations were confirmed, a Multiple Comparison Test (Tukey test) was used to determinate how many means were different between them (Zar, 1984).

Finally, the possible relationship between the different variables (heavy metals and organic matter content) was estimated by determining the correlation coefficients. A high correlation coefficient implies a strong relationship between the variables and, although it is not a measure of quantitative change of one variable with respect to the other, it is a measure of intensity of association between the two variables (Zar, 1984). All statistical analyses were performed with the software Statistica for Windows 4.0 (Statsoft, Inc.).

3. Results and discussion

3.1. Heavy metals in the AOTS sediments

The spatial and temporal distributions of Zn, Cu and Pb in the sediments are represented in Figs. 2–4. The

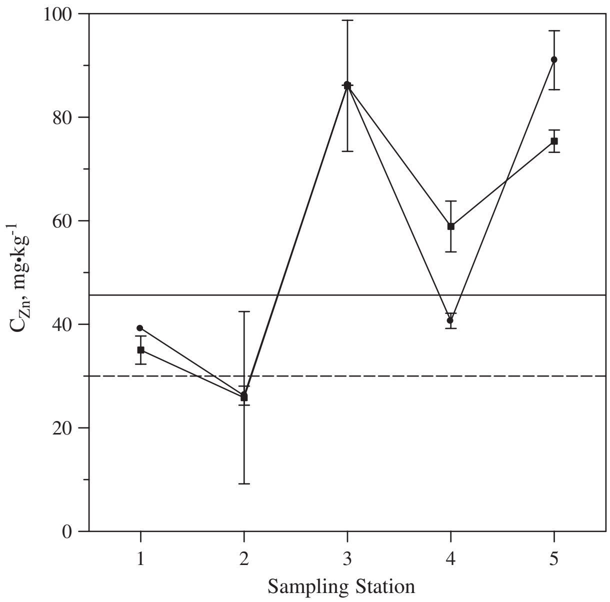

Fig. 2. Concentration of Zn in the sediments of San Pedro River. Error bars indicate standard deviations of two replicates. ●: 1997; ■: 1998; dashed line indicates Zn concentration in sandstone. Solid line indicates Zn concentration in the culture pond.

patterns observed were very similar for every metal studied, with lower concentrations in the zone closer to the mouth, e.g. stations 1 and 2, and higher in the upper zone (stations 3, 4 and 5), where the three aquaculture facilities are located. This trend is consistent with previous results on the water quality of the site (Tovar et al., 2000a).

The average concentrations in sampling stations 1 and 2 were: 30.46 mg kg^{-1} Zn, 4.77 mg kg^{-1} Cu and 9.23 mg kg^{-1} Pb. These two stations are in a pristine area, not affected by aquaculture effluents (Tovar et al., 2000a), and close enough to sampling stations 3–5, to ensure the similarity of the morphology of the sediments of both areas. Besides, the concentrations found in

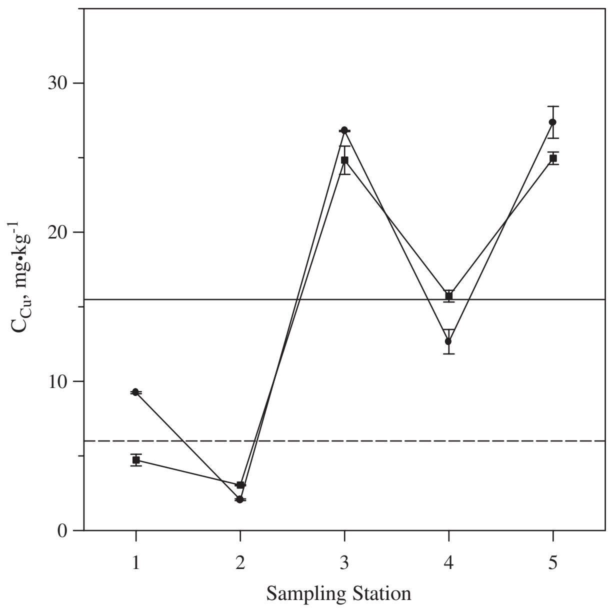

Fig. 3. Concentration of Cu in the sediments of San Pedro River. Error bars indicate standard deviations of two replicates. ●: 1997; ■: 1998; dashed line indicates Cu concentration in sandstone. Solid line indicates Cu concentration in the culture pond.

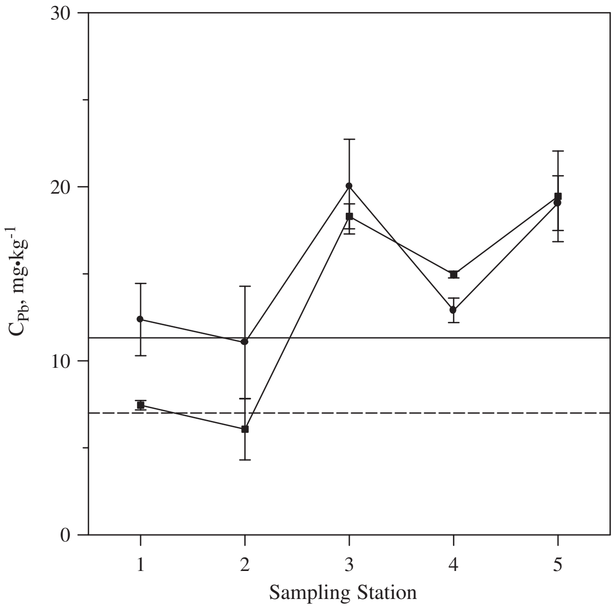

Fig. 4. Concentration of Pb in the sediments of San Pedro River. Error bars indicate standard deviations of two replicates. ●: 1997; ■: 1998; dashed line indicates Pb concentration in sandstone. Solid line indicates Pb concentration in the culture pond.

stations 1 and 2 were very close to the normal concentrations of heavy metals in sandstones (Allard, 1995), usually used as a reference of non-polluted sediments and represented as dashed lines in Figs. 2–4. All these conditions guarantee the correct use of stations 1 and 2 as reference background to quantify the accumulation of metals in stations 3, 4 and 5. Maximum enrichments were 199%, 474%, and 117% for Zn, Cu, and Pb, respectively, with averages of 140%, 362%, and 97%. In contrast to the spatial variability observed, the concentrations obtained for two different sampling campaigns presented, in general, small differences.

As discussed previously, the main pollution source in the AOTS is from aquaculture effluents. In our previous work, we reported loads of suspended solids of up to 30 tons/day from a single fish farm, A in Fig. 1 (Tovar et al., 2000b). In addition, the concentrations of suspended solids in the water column were found to be reduced substantially from stations 3, 4, and 5 to stations 1 and 2, which were of the same order of those in the Bay (Tovar et al., 2000a). This indicates that every day, several tons of solids coming from fish farms settle down on the bottom of the upper part of the AOTS and therefore, the composition of surface sediments changes continuously and it is strongly dependent on the composition of the solids contained in aquaculture effluents, and almost independent on natural processes. Thus, the high concentrations of metals found in marine sediments of the stations 3, 4 and 5 may be attributed to aquaculture. To verify this hypothesis, we measured the direct accumulation of heavy metals in the sediments of an earthen pond of a fish farm (A in Fig. 1) during a culture cycle. Results are discussed in Section 3.4.

3.2. Organic matter in the AOTS sediments

It is well-known that the effluents of any aquaculture facility have very high content of organic matter. Then, if the accumulation of heavy metals in the sediments is related to aquaculture activities, it must be accompanied by an accumulation of organic matter. The concentrations of organic matter measured in the sediments of the studied area during 1997 and 1998 are shown in Fig. 5. The results were very similar to the heavy metals' profiles (see Figs. 2–4) and relatively constant in both sampling expeditions. The lowest values were measured at stations 1 and 2 (1.72% averaged) while the highest contents were determined at stations 3 and 5, corresponding to productive aquaculture sites. Relatively lower concentrations were also measured at sampling station 4, where the Research Center generates lower effluent volumes. The average enrichment in stations 3, 4, and 5 was 445%, with a maximum of 641% in station 3.

3.3. Data treatment

The *p*-values obtained from two-way ANOVA are shown in Table 1. The spatial position of sampling

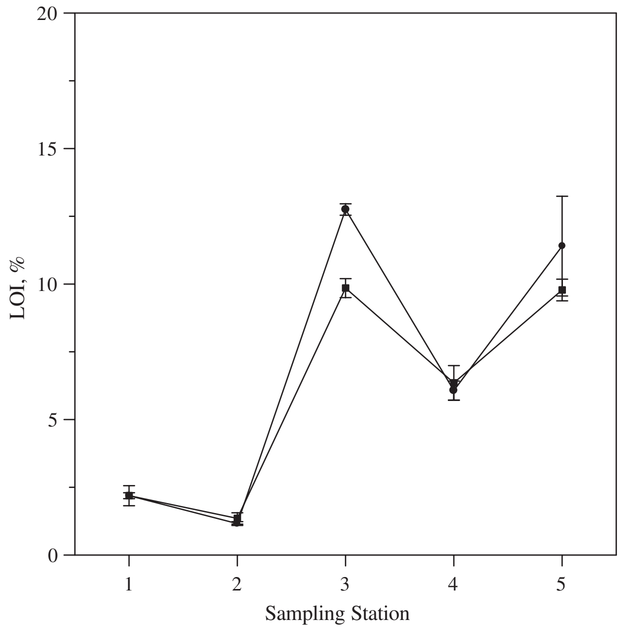

Fig. 5. Contents of organic matter (as Loss on Ignition) in the sediments of San Pedro River. Error bars indicate standard deviations of two replicates. ●: 1997;■: 1998.

station was a significant factor for both heavy metals and organic matter ($p<0.05$). However, significant temporal variations were obtained only for organic matter and Cu concentrations, while the concentrations of Zn and Pb were statistically equal in 1997 and 1998 ($p>0.05$).

Among the most widely accepted multiple comparison tests, we have used the Tukey test, to identify and quantify the influence of the factors space and time for Cu and LOI and only space for Zn and Pb (temporal variations were not significant for these two metals, see Table 1). The results of Tukey tests suggested that Zn and Pb concentrations in stations 1 and 2 were statistically equal ($p>0.05$), and concentrations in station 1 were also statistically equal to concentrations in stations 4. Besides, the concentrations of these two metals in stations 3 and 5, where the main aquaculture effluents are discharged, were statistically equal between them, but different from the other three sampling sites.

In the case of Cu and organic matter, both spatial and temporal factors have to be taken into account to apply the Tukey test. Although the results obtained for Cu (see also Fig. 3) seem very similar to those obtained for the other metals, the very high precision typically achieved in copper measurement by atomic absorption spectroscopy implies that very small differences between concentration means may become statistically significant. Spatial distribution of Cu in the sediments of the AOTS can be considered, in general, similar to Zn and Pb distributions. Besides, significant temporal variations were obtained for sampling stations 1, 4 and 5. These variations, as seen in Fig. 3, are very small, and even negative in stations 1 and 5, and thus, no temporal accumulation of Cu can be established from the results obtained. The absence of temporal variations in the concentration of metals in the sediments is in agreement with the assumption of equal composition of recent sediments and solid matter originated in the fish farms, which always has almost the same composition.

The results of Tukey test for organic matter were very similar to those obtained for metals. Thus, stations 1 and 2 (non-affected area) were statistically equal, as well as stations 3 and 5 (affected area).

Once the temporal and spatial variations of heavy metals and organic matter have been established, we evaluated the possible correlations among the concentrations of each heavy metal and organic matter in the sediment. The results obtained are shown in Table 2, suggesting high correlations (r positive and close to 1) between all the variables. Although these correlations do not imply cause and effect, they give another

Table 1
p values for the ANOVA of heavy metals and organic matter contents in the sediments of San Pedro River

Factor	Zn	Cu	Pb	LOI
Factor 1: temporal	0.821	0.003	0.056	0.008
Factor 2: spatial	0.000	0.000	0.000	0.000
Interaction: 1*2	0.086	0.000	0.087	0.029

Table 2
Correlation coefficients for heavy metals and organic matter (as LOI) in the sediments of San Pedro River

Zn	Cu	Pb	LOI	
*	0.98	0.93	0.97	Zn
	*	0.95	0.99	Cu
		*	0.94	Pb
			*	LOI

evidence that the accumulation of heavy metals in the sediments of the aquaculture zone (especially sampling stations 3 and 5) are caused by the activities of the fish farms.

3.4. Heavy metal in the pond sediments

At the beginning of culture cycle (March, 1997), metals' concentrations in the sediments of a clean pond were 11.41 mg kg^{-1} Zn, 5.45 mg kg^{-1} Cu and 6.18 mg kg^{-1} Pb. At the end of the culture cycle (April 1999) a superficial layer of sediments was formed in the bottom of the pond from the settlement of solids during fish culture (uneaten food and faeces). The concentrations of metals in these sediments were 45.64 mg kg^{-1} Zn, 15.49 mg kg^{-1} Cu and 11.32 mg kg^{-1} Pb. These final concentrations are represented, as solid lines, in Figs. 2–4 and, as can be observed, are higher than background concentrations (sampling stations 1 and 2) and closer to values found in stations 3, 4, and 5. In fact, as a direct consequence of marine aquaculture, increments of about 300%, 184% and 83% for Zn, Cu and Pb, were observed in the sediments of the pond. If we compare the ratios between metal concentrations (Pb : Cu : Zn) in the sediments of the culture pond (1 : 1.37 : 4.04) and in the average values of sampling stations 3, 4 and 5 (1 : 1.21 : 4.04), we can confirm that the composition of both sediments is practically identical, thus suggesting the same origin, and are different from the values (1 : 0.52 : 3.30) obtained in the clean zone of the AOTS. This indicates that aquaculture activities are responsible for high concentrations of Zn, Cu and Pb found in the sediments of the upper part of the San Pedro River.

The effect of the aquaculture activities on the accumulation of heavy metals in the sediments is reinforced by calculating the net metal amounts discharged in the river by aquaculture effluents. Farm A discharges an average of 4522 tons/year of suspended solids (Tovar et al., 2000b). If we assume that discharged solids and superficial sediments of the culture pond have the same composition we obtain annual discharges of 207.8 kg Zn, 70.5 kg Cu and 51.5 kg Pb.

Several previous studies described the accumulation of heavy metals as zinc and copper in marine sediments caused by fish culture. Chou et al. (2002), reported high levels of Cu and Zn in sediments related to Canadian fish farms. Uotila (1991) attributed the accumulation of zinc to fish food, while copper accumulation was mainly related to the paintings used in culture cages. However, the accumulation of copper has been also reported for bivalves' culture, where paintings are not employed (Han et al., 1996). In our case, because fish food is the only input of the system during the culture, they should content significant concentrations of Zn, Cu and Pb. To confirm this fact, we have analyzed metal concentrations of two different fish feeds used in farm A.

3.5. Heavy metals in fish food

Both samples analyzed presented high levels of Zn and Cu (Zn: 180.5 and 168.0 mg kg^{-1}; Cu: 10.1 and 8.93 mg kg^{-1}, respectively) and relatively lower concentration of Pb (0.25 and 0.43 mg kg^{-1}). High concentrations of Zn and Cu in fish food have been previously described (Uotila, 1991; Serra et al., 1996). These values are in good agreement with the higher accumulation of Zn and Cu observed in the sediments of the study zone, and the lower for Pb.

To evaluate the discharge of heavy metals from the farms, we must realize that, for costs reduction, the producers calculate the amount of food given to fishes very accurately and then, suspended solids present in the effluent are mainly formed by the faeces of the fishes plus a small amount of uneaten food. The bioaccumulation of heavy metals by fishes is: $Zn \gg Cu > Pb$ (Al-Ghais, 1995; Powell et al., 1981). Thus, the solids discharged to the environment are enriched in Cu and Pb and depleted in Zn, if compared to fish feed. On the other hand, although some alterations may be found (Chapman et al., 1998) the affinity of the divalent heavy metals by organic matter and particles in sediments mostly follows the Irving–Williams series, i.e.: $Pb > Cu > Zn$ (Horowitz, 1991, Irving and Williams, 1952). These facts can explain why the sediments of the affected stations have higher relative enrichment for Cu (362%) than for Zn (140%) and, the relatively high percentage of accumulation of Pb in the sediments of the river (97%).

4. Conclusions

The results obtained demonstrate that the enrichment of Zn, Cu and Pb in the sediments can be attributed to marine aquaculture. As mentioned in the Introduction section, this fact has been previously reported in a few works (Uotila, 1991; Urdaneta, 1995; De Gregori et al., 1996; Carbonell et al., 1998). Among them, only Uotila selected a clean station to be used as reference. Although this author did not report the accumulation of lead, he described average accumulations of copper (380%) and zinc (167%) very similar to those obtained by us (362% and 140%, respectively). Recently, it has appeared in a study on the accumulation of Cu, Zn, Fe, Mn and organic carbon in sediments of aquaculture areas where important accumulations of Cu (56.7–306.7%), Zn (45.3–414.2%) and organic matter (12.5–468.8%) were reported (Chou et al., 2002).

To establish the importance of the metal accumulation for the ecosystems, we have compared our results with the values recommended by Canadian Council of Minister of the Environment in *Canadian Sediment Quality Guidelines for the Protection of Aquatic Life* (CCME, 1999). Although the evaluation of heavy metal levels in sediments by comparison with guidelines established for different zones has to be done just like an approximation, we have chosen this guideline because it is recent, and focused to the protection of aquatic life. The highest concentrations of zinc and lead measured in the sediments of the San Pedro river were below the recommended value in all the cases (124 and 34.2 mg kg^{-1}, respectively). However, the concentrations of copper determined at sampling stations 3 and 5 were above guideline concentration (18.7 mg kg^{-1}) but they were below the probable effects level (108 mg kg^{-1}). We can conclude that marine aquaculture can increase the levels of organic matter and heavy metals in the sediments but, in the case of the San Pedro River, the levels measured in 1997 and 1998 were not dangerous for the aquatic life of the ecosystem.

Acknowledgement

The collaboration of the Servicio Central de Ciencia y Tecnología (División de Espectroscopía) of the University of Cádiz is acknowledged, with special mention to Mr. Antonio Benítez.

References

Al-Ghais, S.M., 1995. Heavy metal concentrations in the tissue of *Sparus sarba* Forskål, 1775 from the United Arab Emirates. B. Environ. Contam. Toxicol. 55, 581–587.

Allard, B., 1995. Groundwater. In: Salbu, B., Steinnes, E. (Eds.), Trace Elements in Natural Waters. CRC Press, Boca Raton, p. 155.

Belias, C.V., Bikas, V.G., Dassenakis, M.J., Scoullos, M.J., 2003. Environmental impacts of coastal aquaculture in eastern Mediterranean bays. The case of Astakos Gulf, Greece. Environ. Sci. Pollut. Res. 10, 287–295.

Carbonell, G., Ramos, C., Tarazona, J.V., 1998. Metals in shrimp culture areas from the Gulf of Fonseca, Central America. I. Sediments. B. Environ. Contam. Toxicol. 60, 252–259.

CCME (Canadian Council of Ministers of the Environment), 1999. Canadian Environment Quality Guidelines. Canadian Council of Ministers of the Environment, Winnipeg, Manitoba, Canada. http://www.ec.gc.ca/ceqg-rcqe/sediment.htm.

Chapman, P.M., Wang, F., Janssen, C., Persoone, G., Allen, H., 1998. Ecotoxicology of metals in aquatic sediments: binding and release, bioavailability, risk assessment, and remediation. Can. J. Fish. Aquat. Sci. 55, 2221–2243.

Chou, C.L., Haya, K., Paon, L.A., Burridge, L., Moffatt, J.D., 2002. Aquaculture-related trace metals in sediments and lobsters and relevance to environmental monitoring program ratings for near-field effects. Mar. Pollut. Bull. 44, 1259–1268.

De Gregori, I., Pinochet, H., Gras, N., Muñoz, L., 1996. Variability of cadmium, copper and zinc levels in mollusks and associated sediments from Chile. Environ. Pollut. 92, 359–368.

Han, B.C., Jeng, W.L., Hung, T.C., Wen, M.L., 1996. Relationship between copper speciation in sediments and bioaccumulation by marine bivalves. Environ. Pollut. 91 (1), 35–39.

Horowitz, A.J., 1991. Sediment-Trace Element Chemistry. Lewis Publishers, Boca Raton.

Horowitz, A.J., Elrick, K., 1987. The relation of stream sediment surface area, grain size, and composition to trace element chemistry. Appl. Geochem. 2, 437–451.

Irving, H.M., Williams, R.J.P., 1952. Some factors controlling the selectivity of organic reagents. Analyst 77, 813–829.

Jackson, M.L., 1982. Análisis Químico de Suelos, 4th Edition in Spanish. Omega, Barcelona.

Jambrina, M.C., 1999. Problemática medioambiental de la acuicultura marina. Propuestas de medidas correctoras. In: Pérez, A., Zamora, S., Lucena, J. (Eds.), Contaminación marina: orígenes, bases ecológicas, evaluación de impactos y medidas correctoras. Aulas del Mar, Universidad de Murcia, Murcia, pp. 335–389.

Kennish, M.J., 1997. Estuarine and Marine Pollution. CRC Press, Boca Raton.

Luoma, S.N., 1990. Processes affecting metal concentrations in estuarine and coastal marine sediments. In: Furness, R.W., Rainbow, P.S. (Eds.), Heavy Metals in the Marine Environment. CRC Press, Boca Raton, p. 124.

Otero, X.L., Vidal-Torrado, P., de Anta, R.M.C., Macias, F., 2005. Trace elements in biodeposits and sediments from mussel culture in the Ria de Arousa (Galicia, NW Spain). Environ. Pollut. 136, 119–134.

Powell, J.H., Powell, R.E., Fielder, D.R., 1981. Trace element concentrations in tropical marine fish at Bougainville Island, Papua New Guinea. Water Air Soil Pollut. 16, 143–158.

Schendel, E.K., Nordstrom, S.E., Lavkulich, L.M., 2004. Floc and sediment properties and their environmental distribution from a marine fish farm. Aquac. Res. 35, 483–493.

Serra, R., Isani, G., Catan, O., Carpené, E., 1996. Effects of different levels of dietary zinc on the Gilthead, *Sparus aurata*, during the growing season. Biol. Trace Elem. Res. 51, 107–116.

Tovar, A., Moreno, C., Mánuel-Vez, M.P., García-Vargas, M., 2000a. Environmental impacts of intensive aquaculture in marine waters. Water Res. 34, 334–342.

Tovar, A., Moreno, C., Mánuel-Vez, M.P., García-Vargas, M., 2000b. Environmental implications of intensive marine aquaculture in earthen ponds. Mar. Pollut. Bull. 40 (11), 981–988.

Uotila, J., 1991. Metal contents and spread of fish farming sludge in southwestern Finland. Marine Aquaculture and Environment. Valtion Painatuskescos Oy, Helsinki, pp. 120–125.
Urdaneta, H., 1995. Contenido total de Cd, Cu, Cr, Mn, Hg, Ni y Pb en sedimentos superficiales de una estación de piscicultura del municipio Páez, Estado Zulia, Venezuela. Bol. Cent. Investig. Biol. 29 (1), 1–16.
Zar, J.H., 1984. Biostatistical Analysis. Prentice-Hall, Englewood Cliffs.