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Sediment Contamination and Toxicity in the Guadalquivir River (Southwest, Spain)

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Abstract: A segment of the Guadalquivir River was assessed between the Alcalá del Río dam and Seville through an integrative sediment quality assessment. Chemical concentrations of metals and toxicity under laboratory conditions were used as lines of evidence. A battery of bioassays with four organisms (the amphipod *Ampelisca brevicornis*, the bacteria *Vibrio fischeri*, the sea urchin *Paracentrotus lividus*, and the oligochaete *Tubifex tubifex*) exposed to sediment made it possible to determine the potential risk associated. The sediments from Seville and Alcalá del Río showed higher values of the concentration of most metals than the Algaba station, with Cu (35–37 μg/g), Zn (70–75 μg/g), Ni (23–26 μg/g), and Pb (27–30 μg/g) being the most abundant metals. An increasing toxicity gradient was shown downstream among the bioassays with the amphipod *A. brevicornis*, the fertilization test using the sea urchin *P. lividus*, and the freshwater worm growth *T. tubifex*. Conversely, an increasing toxicity gradient was shown upstream in the embryo-larval *P. lividus* development. The link between sediment contamination and toxicity makes it possible to obtain a gradient of contaminant concentration comparable with nationally and internationally widely accepted sediment quality guidelines in order to establish the risk associated with this area of study.

Keywords: lines of evidence; sediment quality guidelines; Guadalquivir River; toxicity tests

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1. Introduction

The Guadalquivir River (SW, Spain) is the second longest river in Spain and the only one which allows navigation in the Estuary, being the main water source of a Spanish region with more than 7 million inhabitants. It reaches the Gulf of Cádiz (Atlantic Ocean), bordering Doñana National Park Reserve which is the most important wintering site for many birds in Europe. However, this region has been exposed for centuries to mining activities which have greatly enriched the metal content of its water and soils [1].

Integration of chemical concentration and toxicity responses is the best way to determine the sediment [2,3]. It is known that sediments are able to transport contaminants much more easily than water [4], but also act as a sink and/or source of contaminants [5]. Therefore, biological effects based on laboratory tests can determine toxic responses. Sediment bioassays are simple tests that evaluate the responses of the tested organism to contaminated sediments under controlled conditions [6]. Despite of the fact that some reports have pointed out that biological tests or bioassays are decisive to understand the toxic effects of sediments [7–9], the final decision of the sediment status is based on a comparison between levels of contaminants measured in the sediments against different sediment quality guidelines (SQGs).

A battery of tests was selected using different trophic levels to determine sediment toxicity: (1) a whole sediment survival bioassay using the amphipod *Ampelisca brevicornis*,

(2) the commercial Basic Solid Phase Test (BSPT) of Microtox® based on the bioluminescence of the bacteria *Vibrio fischeri*, (3) the *Paracentrotus lividus* sea urchin fertilization success and (4) embryo-larval development bioassays, and (5) a whole sediment toxicity test with the oligochaete worm *Tubifex tubifex*.

The acute bioassay with the *A. brevicornis* amphipod has shown this species as a sensitive organism valid to assess the toxicity of contaminated sediments [10–14]. The commercial bioassay Microtox® has been used to detect the "hot spots" of field contamination in the screening procedure [15,16], and also to assess oil contaminated sediments [11,17,18]. Sea urchin toxicity tests use fertilization and larval development as endpoints, which are considered to be useful tools for assessing toxicity in marine environments. These acute toxicity tests have been applied to determine the toxicity of sediments [19–21] under laboratory conditions, and have been included and standardized by several national environmental agencies [22,23].

The chronic toxicity test with the oligochaete *T. tubifex* was widely used for sediment bioassessment in the Great Lakes of North America and included in environmental programs [24,25]. In Europe, *T. tubifex* was also used for sediment toxicity assessment [26–28], as well as in bioaccumulation studies [3,29] and organic compounds [30–32]. The followed procedure was developed by [26], and recommended in the American Society for Testing Materials (ASTM) guide [33]. Therefore, they are considered ideal for determining the toxicity of contaminants [34–36] in freshwater sediments. This bioassay was selected to assess toxicity during low tide when salinity falls down, since the tidal influence comes up to the Alcalá del Rio dam (maximum recorded high tide: 1.70 m).

The main aim of this work is to characterize the sediment quality in different areas of the Guadalquivir River affected by different levels of contamination by linking both chemical concentrations and bioassay responses using a weight of evidence approach.

2. Materials and Methods

2.1. Study Area

The present study was performed in the lower course of the Guadalquivir River, between the Alcalá del Río dam and the city of Seville (Figure 1). The study areas are shown in Figure 1: (1) Alcalá del Río town (AR), 1 km downstream the dam; (2) Algaba (AL) municipality, 6 km downstream from the AR station; and (3) Seville (SE) city, 6 km downstream from the AL station, Seville being the main urban and industrial center of this area. According the WFD criteria, the Waterbody Manager of the Guadalquivir River established as "moderate status" the river course from the AR dam to the SE [37]. These sites were selected to test the influence of the historic mining activity [1]. The local water body manager [37] identified different diffuse sources of contaminants from urban, industrial, and agricultural waste waters as the key pressure on Guadalquivir river waters.

Two more sites were chosen as control stations: (1) San Pedro river (RSP), which was selected for the amphipod, the BSPT of Microtox®, and the sea urchin fertilization and embryo-larval bioassays, as well as the fact that it has been previously determined as a clean area [38,39], and (2) Barazar (BAR) for the chronic toxicity test with *T. tubifex* due it's proved quality as a control for this species in particular [40].

2.2. Sediment Sampling

Surface sediment samples (5–10 cm) were collected at the three described sites along the Guadalquivir River (AR, AL and SE) and the control site (RSP) with a 0.025 m^2 Van Veen grab and transferred to a plastic bucket. Sediment was transported in a cooler to the laboratory. There, the contents of the bucket were homogenized, and subsamples were destined for physical-chemical characterization and used for toxicological tests. They were kept at 4 °C in the dark.

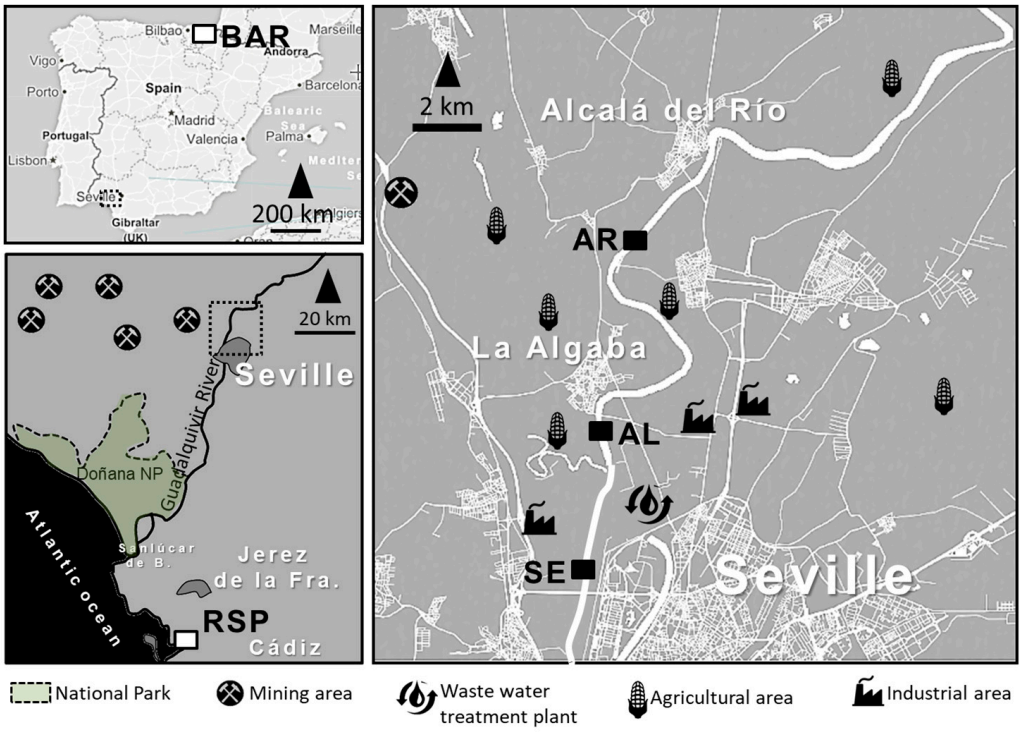


Figure 1. Location of sampling stations in the Guadalquivir River: Alcalá del Río town (AR), Algaba (AL) municipality, and Seville (SE); and controls from Río de San Pedro (RSP) in Cádiz, and Barazar (BAR) in Bilbao.

2.3. Chemical Analyses

Sediment aliquots for chemical analysis were dried at room temperature and then gently homogenized. Organic carbon content was determined by using the method of [41]. Aliquots of sediments were acid-digested (65% H_2NO_3 and 33% H_2O_2) in closed vessels in triplicate. Metals Cu, Zn, Fe, Co, Ni, Cd, and Pb, and the metalloid As were determined using ICP-MS, ICP-AES, and GF-AAS. The concentration was calculated on a dry weight basis. Reactive blanks and reference materials (CRM478 BCR) were used to ensure quality control.

2.4. Toxicity Tests in Laboratory

As mentioned above, sediments from the analyzed points were used to perform a battery of acute toxicity tests in laboratory conditions: (1) a whole sediment survival bioassay using the *A. brevicornis* amphipod, (2) the commercial BSPT of Microtox®, (3) the sea urchin fertilization success and (4) embryo-larval development bioassays, and (5) a whole sediment toxicity test with *T. tubifex.*

2.4.1. Amphipod Toxicity Test

The collection and acclimation of organisms, the collection and preparation of sediment, and the *A. brevicornis* toxicity test procedure were conducted following the methodology pointed out by different authors and standard protocols [42,43]. Water quality and the amphipods' mortality was determined. Results are expressed as survival of organisms after 10 days of exposure.

2.4.2. Bacterial Toxicity Test

The decrease of the bioluminescence of the bacteria *V. fischeri* (Microtox® BSPT) is used as a quality indicator. This bioassay was conducted with the commercial Microtox®(model 500) following the Basic Solid Phase Test (BSPT) according to the standard operating procedure [44] with the modifications reported by [10,45]. Calculations to obtain this

concentration were performed with the Microtox Omni® (Version 1.18) software of Strategic Diagnostics Inc. Results were expressed as an IC50 (mg L^{-1}) value, which is the concentration of dry sediment where the luminescence is reduced by half.

2.4.3. Sea Urchin Toxicity Tests

Adults of sea urchins *Paracentrotus lividus* were collected by free diving from an Andalusian beach near to El Estrecho Natural Park. They were placed in a 30 L plastic cooler and transported to the laboratory, where they were acclimated in chambers with aerated clean water at 15 °C for 10 days. Sediment elutriates were obtained using a modification of the [46] method.

Paracentrotus lividus fertilization test: gametes used for fertilization were obtained from a single pair by injection of 1 mL KCl (0.5 M) through the peri-oral membrane. The fertilization procedure followed the one described by [47], adapted from [48]. Each sample, plus the control containing clean water, was run per triplicate.

Paracentrotus lividus embryo development test: The same pools of gametes obtained during the fertilization test were used for the larval development test. The in vitro embryo-larval bioassay was developed using the sea urchin larvae after 48 h of exposition to sediment elutriates. Fecundation and test conditions followed the protocol developed by [49].

The endpoint recorded was the percentage of embryological success, expressed as the percentage of normal pluteus (well-developed four-arm embryos, according to the criteria reported by [47], and calculated by counting 100 larvae per vial with an optical microscope (OLYMPUS CKX41)).

2.4.4. Oligochaete Chronic Toxicity Test

The chronic sediment toxicity test with annelid *Tubifex tubifex* was performed following [33,49,50] protocols and modifications [26,40]. Worms obtained from the Natural Park of Gorbeia (Vizcaya, Spain) were exposed for 28 days to the different sediment samples and a control sediment (called Barazar or BAR) with proved quality [40], which was used for the worm culture.

The endpoints analyzed were growth, reproduction, and mortality after 28 days, calculated respectively as total growth rate (TGR), Total Young Number (TYG), and mortality, following the methodology reported by [40].

2.5. Data Treatment

A one-way analysis of variance (ANOVA) was used to test the significance of the contamination analysis and the toxicity tests compared to control samples, and were followed by multiple comparisons of Dunnett's tests. Statistical analyses of toxicity tests were performed using SPSS 17.0. The acute toxicity for the Microtox BSPT bioassay was analyzed using a gamma model of Microtox Omni software (SDI Europe, Hampshire, UK).

For the analysis of the data from the *T. Tubifex* bioassay, the mortality frequencies in each test sediment were compared with the respective control using Fisher's Exact test. Normality of data and homogeneity of variances were checked through the Shapiro-Wilk and the Levene's F tests, respectively. Data were transformed for normalization when necessary. Single-factor analyses of variance followed by Dunnet's test (if the number of replicates was the same) or a *t*-test with Bonferroni's adjustment (when the number of replicates was different) were used for the comparison of the tests' means and to control for the reproductive and growth variables [43]. When data were not normalized after transformation, the Kruskal-Wallis nonparametric test followed by the Mann-Whitney U test was applied.

The multivariate analysis was performed by means of a factor analysis (Principal Component Analysis (PCA) with varimax normalized rotation). This analysis establishes and quantifies the correlations among the variables from the original data set to facilitate the data interpretation [51,52]. According to [53], only those variables with a loading

of 0.30 or higher were associated with a particular factor. The factor analysis procedure was performed using the STATISTICA software tool (Stat Soft, Inc., Tulsa, OK, USA, 2001; version 6.0). The calculation of SQGs followed the methodology presented in [51] slightly modified by [54].

3. Results and Discussion

Different authors have reported the usefulness of the battery of tests used in this work. For instance, [21] showed that Microtox® BSPT, *A. brevicornis*, and *P. lividus* toxicity tests gave supplementary information about particulate and adsorbed contaminants' effects. Additionally, the present report has developed a standardized toxicity test in freshwater sediments with the oligochaete *T. tubifex* to complete the toxicity information during the salinity decreases in low tide events. The suitability of the different bioassays was confirmed through the validity of the controls.

3.1. Sediment Metals Concentration

Table 1 shows the summarized results of the different contaminants analyzed. No increasing or decreasing pattern downstream was observed. In general, sediments from the AR station showed the highest contaminant content and those from AL the lowest, except for Pb, which was higher at SE and lower at AL sediments. The sediments located in the station close to Seville showed significant differences ($p < 0.05$) with the control station (RSP) for every analyzed contaminant and organic matter.

Table 1. Summarized results of chemical analyses in sediments from the studied stations (Adapted from [55]).

Element		AR	AL	SE	RSP
As	µg/g	10.22	9.77	9.84	7.14
Cd	µg/g	0.27	0.26	0.26	0.13
Co	µg/g	8.63	7.73	7.70	5.82
Cu	µg/g	37.42	35.37	35.51	18.00
Fe	µg/g	23,990	21,920	22,140	15,239
Ni	µg/g	25.42	23.39	23.56	14.00
Pb	µg/g	29.65	27.30	31.73	22.30
Zn	µg/g	75.89	66.79	69.65	49.00
OC	%	1.18	1.06	1.16	0.83

3.2. Toxicity Tests in Laboratory

3.2.1. Amphipod Laboratory Bioassay

Mean mortality results after the 10 day amphipod toxicity test are shown in Figure 2a. The highest mortality (60%) was associated with those organisms exposed to sediments at the SE station. An increasing toxicity gradient was shown downstream, while AR showed the lowest mortality values. Furthermore, stations with the highest toxicity values (SE and AL) showed significant differences ($p < 0.05$) compared to the control station (RSP).

3.2.2. Microtox® Bioassay

The highest inhibition of bioluminescence, which corresponds to an $IC_{50} < 1000$ mg L^{-1} dry weight, is shown in the samples collected at SE (Figure 2b). Therefore, this station was only under the limit assumed for sediment toxicity (1000 mg L^{-1} dry weight) according to the Canadian standards [23]. On the other hand, the sediments obtained from the RSP, and used as a control station, showed the highest values of IC_{50} (12,186.67 mg L^{-1} dry weight). No significant differences ($p < 0.05$) were observed between each station and the control.

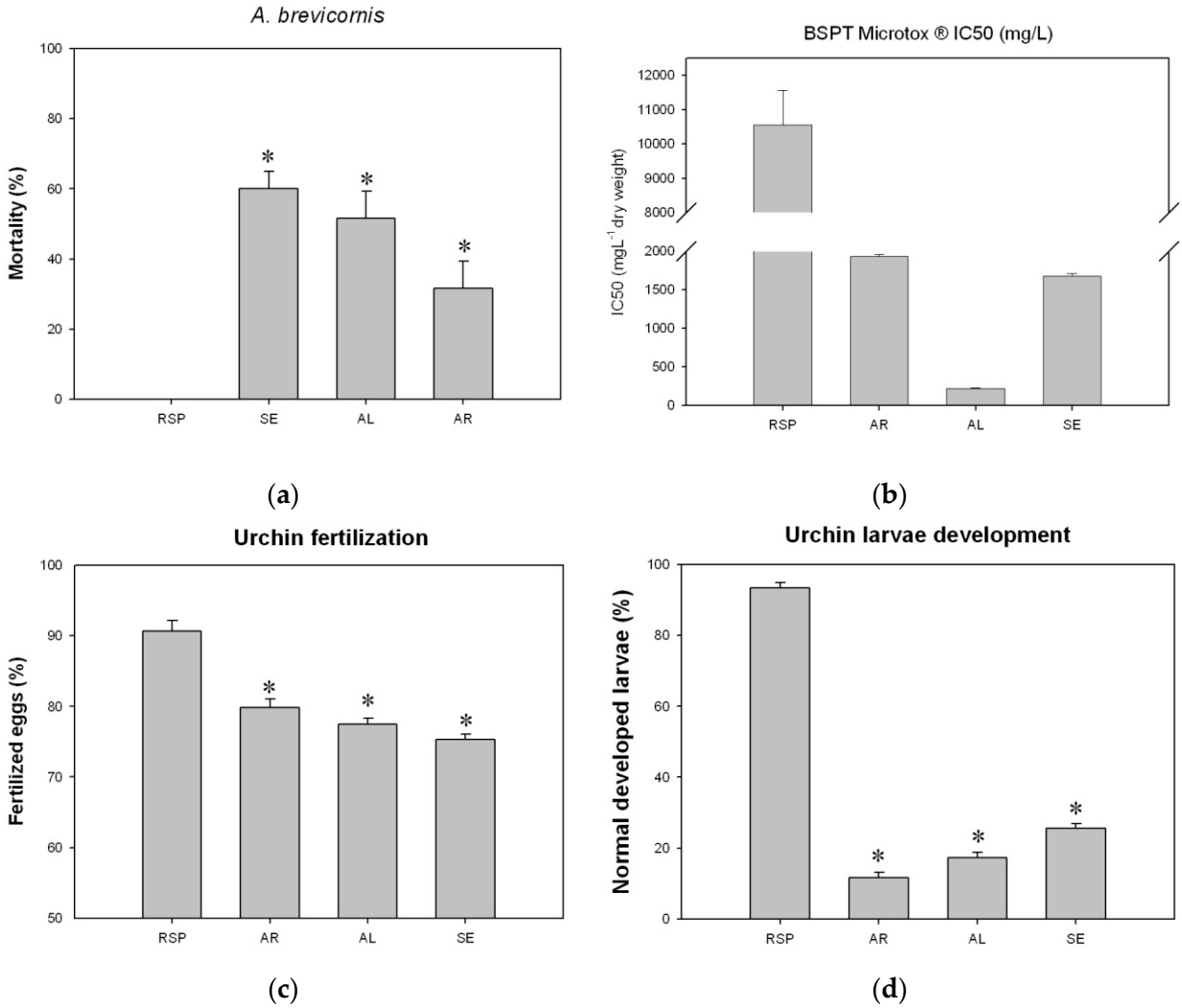


Figure 2. (**a**) *A. brevicornis* mortality (%) after 10 d exposure. (**b**) IC_{50} results (mg L^{-1} dw sediment) obtained from the BSPT Microtox® samples. (**c**) Results of the *P. lividus* fertilization bioassay test: average percentage of fertilized eggs after exposure of the sperm to the sediment samples under laboratory conditions. (**d**) Results of the *P. lividus* embryo-larval bioassay test: average percentage of normal development of larvae after exposure of the fertilized eggs to the sediment samples under laboratory conditions. Asterisk (*) indicates significant differences ($p < 0.05$) between samples and the control (RSP).

3.2.3. Paracentrotus lividus Toxicity Tests

P. lividus Fertilization Bioassay

The percentage of successfully fertilized *P. lividus* eggs in each sampling station is shown in Figure 2c. A gradient of toxicity was observed between the downstream station (SE) with the lowest values of fertilization success and the upstream station AR, which showed the highest values (excluding the control). Significant differences ($p < 0.05$) were observed in all the stations compared with the control (RSP). The control station showed the highest success (more than 90%), confirming the validity of the test.

P. lividus Embryo Development Test

The success percentage of normally-developed *P. lividus* larvae at each station are shown in Figure 2d. An increased number of normal *P. lividus* larvae were observed in the Guadalquivir River stations (AR, AL and SE) and ranged from 12% (AR) to 25% (SE). These results indicated the presence of adverse effects (percentage of normal larvae

development < 80%) and showed significant differences ($p < 0.05$) with the control station (RSP), which showed more than 90% normal larvae development. The results confirm the validity of the test with the RSP station as a control.

Tubifex tubifex Chronic Bioassay

In this bioassay the results of the control station (RSP) also confirmed the validity of the bioassay according to the [33] criteria. Dissolved oxygen was maintained above 60% (except twice: once in the control at 34%, and again in AR at 59%), unionized ammonia (NH_3) was maintained above 1 mg L^{-1}, and pH ranged from 6–8.22 (BAR), 7.59–8.59 (AR), 7.98–8.60 (AL), and 7.85–8.62 (SE).

Figure 3 represents the results for growth (TGR) and reproduction (TYG). A decreasing gradient of growth was shown downstream of the Guadalquivir River. TGR was significantly reduced ($p < 0.05$) in sediments from AL and SE (SE being very close to zero). The TYG was significantly reduced ($p < 0.05$) in the sediments from AR and AL, comparing with the control BAR. Nevertheless, no spatial gradient was shown. Regarding mortality, no significant differences in mortality were observed with respect to the control since mortality was not found in any station.

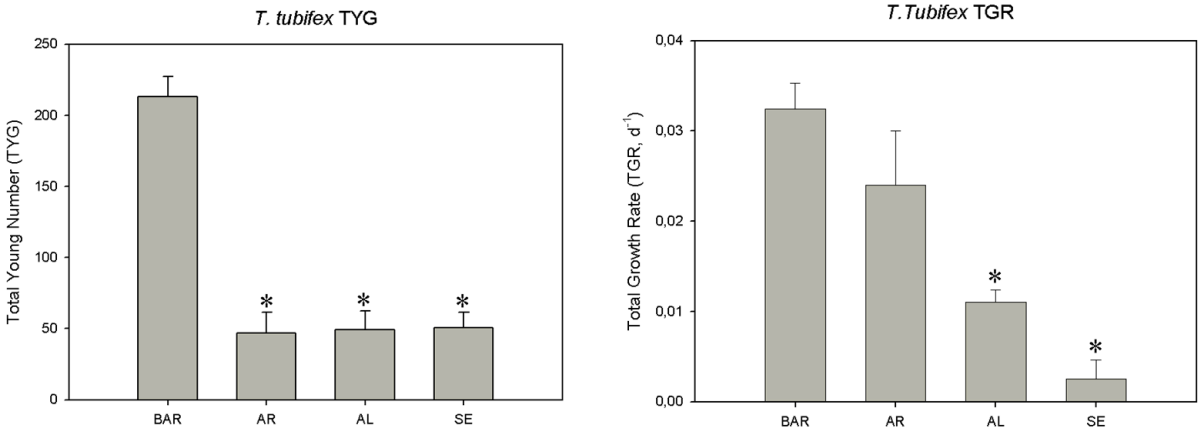


Figure 3. Mean and SD of toxicity endpoints of the *T. tubifex* bioassay: TGR (d^{-1}), TYG, and mortality (%) at each station. Asterisk (*) indicates significant differences ($p < 0.05$) between samples and the control (BAR).

3.3. Linking Contamination and Toxicity

A PCA factor analysis was performed in order to assess the environmental quality of the study area from the Guadalquivir River, employing acute toxicity and chemical analysis. The application of principal component analysis indicated that the original set of variables could be narrowed down to three new factors that explained 100% of the total variance (Table 2).

Factor analysis was performed to link the results of the five bioassays (Microtox® BSPT, *A. brevicornis*, both with *P. lividus*, and *T. tubifex*) with the concentration of the nine contaminants (Cu, Zn, Fe, Co, Ni, Cd, Pb, As, and organic carbon). The first principal factor (F1) explains 78.93% of the total variance and group responses of the sediment toxicity tests (excluding Microtox® and total growth rate measured in *T. tubifex*), metals, and organic carbon analyzed in sediments. However, this low loading of Microtox® in this factor could be due to the fact that this test is a screening bioassay; it is suggested that this biotest by itself may not be representative in certain cases of the full impact of a given pollutant on an ecosystem [17]. The second factor (F2) accounted for 14.31% of the total variance and was correlating the concentration of Cd in sediment with all the responses measured in the bioassays (by presenting positive loadings) except for total young number of *T. tubifex* (which presented a negative value). This correlation between these metals and *T. tubifex* parameters is in agreement with [40], who showed high sensitivity to copper and cadmium. The third factor (F3) accounted for 6.76% of the variance. This factor has

associated contamination by Co to *A. brevicornis* and *T. tubifex* mortality and TYG. Only *A. brevicornis* mortality showed negative loadings. Previous results reported by [13] also related the contamination by Co to the *A. brevicornis* mortality indicating a particular sensitivity of this species to Co.

Table 2. Sorted rotated factor loadings of the original variables for the three principal factors results. Variables selected for the interpretation represented a loading of 0.30 or higher.

Variance %	Factor 1 78.93	Factor 2 14.31	Factor 3 6.76
A. brevicornis mortality	0.764	0.555	−0.327
V. fischeri bioluminescence inhibition		0.954	0.301
P. lividus fertilization	0.881	0.436	
P. lividus embriogenesis	0.917	0.322	
T. tubifex mortality	0.410	0.346	0.843
T. tubifex TYG	0.874	−0.413	0.378
T. tubifex TGR		0.982	
Cu	0.948	0.309	
Zn	0.974		
Fe	0.960		
Co	0.939		0.332
Ni	0.956		
Cd	0.948	0.301	
Pb	0.985		
As	0.955	0.344	
OC	0.998		

A graphical representation of the estimated factor values corresponding to each sampling station is presented in order to confirm the descriptions of these factors (Figure 3).

Through the sediment toxicity test under laboratory conditions, it has been demonstrated that the environmental degradation due to metals in the Guadalquivir river water course can be translated into a potential ecological risk. The study here conducted assessments of the synergetic effect of all the contaminants present in the sediment analyzed. The integration of the results from the different lines of evidence makes it possible to address the extension of the metal contamination and the relationship between these metals (and other potential contaminants not measured) on the effects observed in the toxicity tests conducted.

Based on the multivariate analysis results (Table 2 and Figure 4), different contamination and toxicity processes can be defined at each of the sediments studied here: sediments at station AR show the highest score value for Factor 1, Factor 2 being lowest and negative. The toxicity associated with metals is related to factor 1. This fact can be explained by two different sources of metals and the toxicity of them (Cd, and in lower weight Cu and As, from the negative value of Factor 2). It could be related to the presence of a dam 1 km upstream of the AR sample point that could explain potential phenomena of accumulation and thr release of different metals depending on the season.

Sediments at station AL show the highest value score for Factor 2 and a negative value in Factor 1. Factor 2 includes correlations with toxicity test responses lower than F1 except for Microtox. This could be informative regarding the Microtox® responses in these sediments being lower in the rest of the toxicity tests, and as well as regarding a moderate toxic response in this sediment. The screening nature of this test has been reported [17]. In addition, the moderate toxicity observed in this station is related to a moderate correlation of three metals as well (Cu, Cd, and As), but not to the rest of the analyzed metals. Moreover, different sources of these metals could be included in the explanation of these correlations, and Cu, Cd, and As elements could be part of organic compounds, such as pesticides and fertilizers coming from agriculture activities which have a high economic impact in this area. Furthermore, agriculture has economic importance

in this area as reported by previous work [56]. In addition, the positive value of Factor 3 could confirm this different origin of the contamination of metals and the moderate toxicity observed in these sediments.

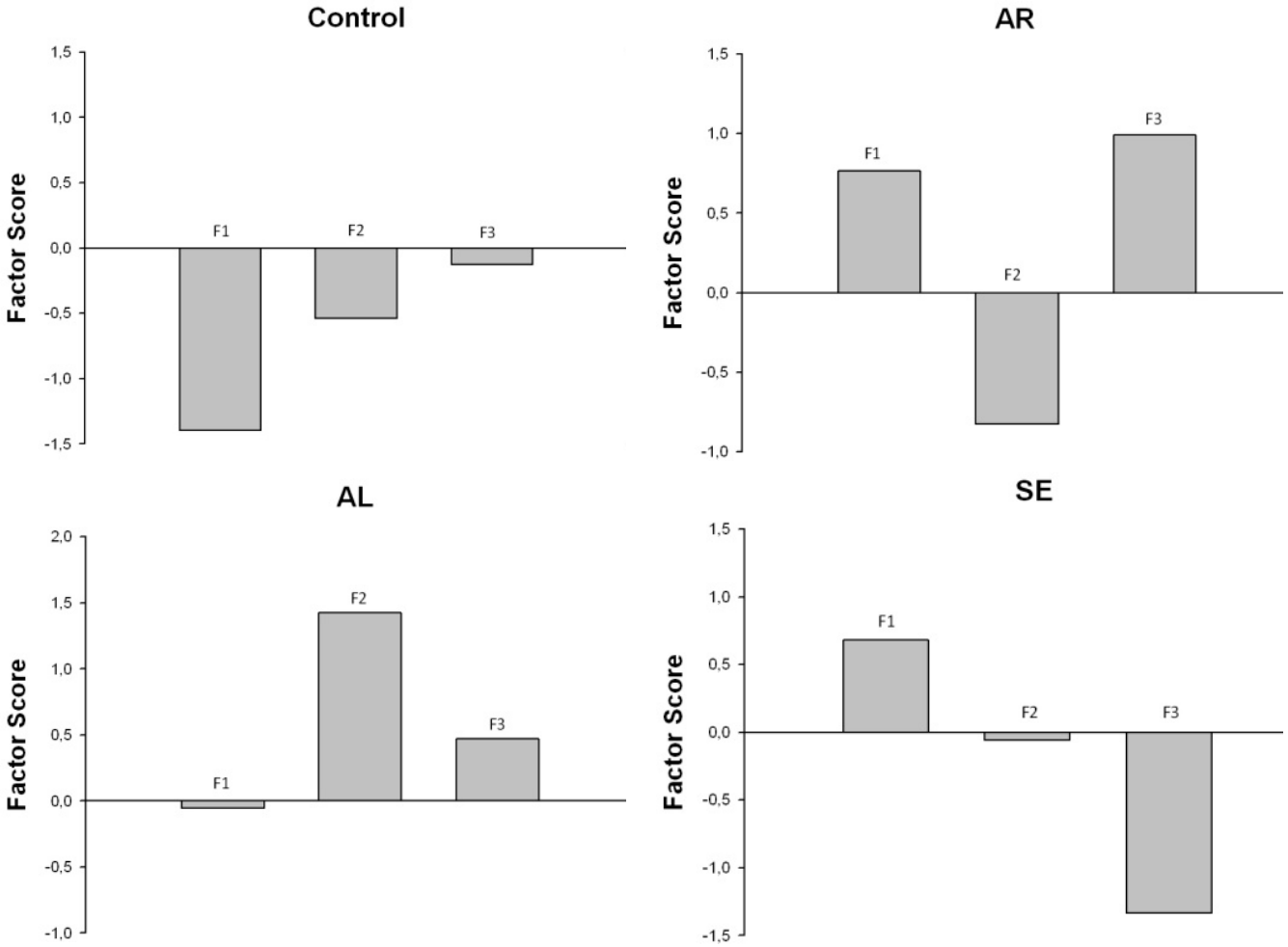


Figure 4. Estimated factor scores from the sampling stations (Control, AR, AL, SE). The factor scores quantify the prevalence of each factor for every station and are established to define each factor.

Sediments at the station located nearby the city of Seville (SE) also show a positive score of Factor 1, informing about the contamination of metals and the toxicity associated with them. However, the values and correlations are lower than station AR and can be considered as intermediate in toxicity in this study. The negative values of Factors 2 and 3 confirm that sediments at this station are less contaminated than those at station AR.

A previous sediment quality assessment was carried out with the biomarker responses of clams in the same stations [55]. AR and AL contamination resulted in the induction of biological stress, and SE contamination (As, Cd, and Cu) caused toxicity as biomarker effects.

From these results, site-specific sediment quality guidelines can be derived for the area studied. These SQGs have been calculated following the procedure reported by [51] and slightly modified by [54]. The SQGs are shown in Table 3.

These values have been compared with nationally and internationally accepted SQGs in order to establish a pollution range in this area, and with the aim of proving the validity of the use of these values to be applied in estuarine environments. In Table 4, this comparison can be observed in detail.

Table 3. Summary of calculated benchmark sediment quality guidelines (SQGs; mg kg^{-1} dw) proposed to evaluate sediment quality in the Guadalquivir River course between the Alcalá del Rio and Seville for the metals Cu, Zn, Fe, Co, Ni, Cd, and Pb, and the metalloid as associated with toxic effects.

Contaminant	Sediment Quality Guidelines (SQGs)		
	Not Polluted	Moderately Polluted	Highly Polluted
Cu	≤35.37	35.37–35.51	≥35.51
Zn	≤66.79	66.79–69.65	≥69.65
Fe	≤21,920	21,920–22,140	≥22,140
Co	≤5.82	5.82–7.70	≥7.70
Ni	≤23.39	23.39–23.56	≥23.56
Cd	≤0.13	0.13–0.26	≥0.26
Pb	≤27.29	27.29–28.40	≥28.40
As	≤9.77	9.77–9.84	≥9.84

Table 4. SQGs comparison for marine sediments.

	CCME [1]		Riba [6]		CEDEX [2]			Present Study
	ISQG	PEL	V1	V2	AL1	AL2	AL3	ALR
As	7.24	41.6	27.4	213	35	70	280	9.77–9.84
Cd	0.7	4.2	0.51	0.96	1.20	2.40	9.60	0.13–0.26
Cu	18.7	108	209	979	70	168	675	35.37–35.51
Ni			-	-	30	63	234	23.39–23.56
Pb	30.2	112	260	270	80	219	600	27.29–28.4
Zn	124	271	513	1310	205	410	1640	66.79–69.95

[1] [55,57]; [2] [58], ISQG: interim marine sediment quality guidelines; PEL: Probable effect levels.

Comparing the results from this study with the different widely accepted SQGs, it is shown that not one of the metal concentrations founded in the Guadalquivir River fit with the action level proposed by these SQGs. However, the action level established in this study was proposed according to several laboratory bioassays, demonstrating sediment toxicity in all the cases with irreversible ecological effects.

The SQGs calculated in the present study are in the interval of the international guidelines for all the metals [57]. Only Zn and Pb showed lower values for the guidelines than the rest of the international proposed guidelines. Ni showed guideline values close to the lower guidelines proposed by [58]. The metals Cu, Cd, and As, showed values between the lowest and highest guideline values proposed by other authors (Table 4). From these values it could be suggested that the toxic effects determined in the area are associated with the lowest SQGs proposed by other authors.

4. Conclusions

Sediments from the Guadalquivir River were assessed through a battery of bioassays with amphipods, sea urchins, bacteria, and worms in laboratory conditions. Based on the results obtained for metal contamination and toxicity assessment, the present study demonstrated alterations associated with the contamination measured in sediments. Sediments from the AL and SE stations showed an important relationship between metals and toxic endpoints which are higher in AL than in the area of SE. Sediments at the AR station showed a complex mixture of sources of metals with a moderate effect compared to the other two stations with a potentially different origin of Cd, Cu, Co, and As at the location of the dam upstream of the estuary.

A sediment quality guideline for metals analyzed was calculated and compared to international and national guidelines showing the action level gap between them, with a contamination range higher than those from this study. For this reason, this set of chemical guidelines derived based on toxic studies could be useful for future river-

ine/estuarine/coastal management plans. The approach used in this study by means of different lines of evidence (contamination of metals and sediment toxicity) is a first step of sediment quality assessment in the area. Similar studies including other contaminants (organics and an emergent like pharmaceuticals) will improve the integrated analysis for a correct sediment quality assessment in the area.

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