Universidad del País Vasco
Euskal Herriko Unibertsitatea

Metalak eta metaloideak itsasadarretan : Kutsadura jarraitzeko erreminta analitikoen garapena

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SARRERA OROKORRA

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Hitzaurrea

Bilboko hiriak eta honen inguruko lurraldeak beti izan dute harreman berezia bere itsasadarrarekin. Industrializazioa baino lehen herrien arteko komunikazio naturala zen itsasadarra. Beranduago, hiriaren erabateko garapen ekonomikoa ekarri zuen burdinaren garraiabide (gure meategietatik Ingalaterrara) bihurtu zen. Baliabideen sorburu bukaezina zirudien itsasadarra. Baina gizakiok, ohiko modura, errespetu gutxiz ordain eskasa eman genion trukean. Aberastasuna eman zigun ingurunea babestu eta zaindu beharrean zikindu eta kaltetu besterik ez genuen egin, aberats bidean sortutako soberakinak han eta hemen zabalduz, inolako kontrolik gabe, Bizkaitarrontzat betidanik altxorra izan zena hiltzeko zorian utziz.

Gaixo baten moduan gehiegikerien ondorioak jasan zituen itsasadarrak, hots, kutsatzaileen gehiegizko kontzentrazioek eragiten duten dardarka etengabea eta oxigeno-eskasiaren ondoriozko bizitzaren pultsuaren ahultasuna, besteak beste. Zorionez, diagnosia ez zen beranduegi heldu eta martxan jarritako tratamenduak espero zitekeen eragina izan zuen gaixoarengan, bere aurpegia, pixkanaka pixkanaka, kolorea berreskuratzen hasi zelarik.

Gaixoa, alabaina, sendatzeko prozesuan dago oraindik, egunetik egunera bere itxura hobetuz doalarik. Ez dugu lokartu behar, beraz, eta tratamenduarekin aurrera jarraitu behar dugu, gaixoaren bilakaera hurbiletik jarraituz. Gure lan-taldea lan horretan ibili da azken urtetan. 2005ean hasita eta 2011. urtera bitartean, puntual hurbildu gara hiru hilero gaixoarengana, medikuak odola eta gernua hartzen dituen moduan, guk ur- eta sedimentu-laginak hartzera, bertan osasunari hain kalte larria egiten dioten traza mailako metal eta metaloideen kontzentrazioak neurtuz. 2005etik 2008ra egindako frogen erregistroak ikertaldearen beste partaide batek bere Doktorego-Tesian aurkeztu zituen[1], eta oraingo lan honetan 2008tik 2011ra doazenak bildu dira.

Estiloz aldatuz eta terminologia zientifikoagoa erabiliz, lan honen berezitasunak eta helburuak azalduko dira ondorengo lerroetan.

Fdez-Ortiz de Vallejuelo-k 2005eko urtarriletik 2008ko urtarrilera buruturiko lanari segida eman zaio Doktorego-Tesi honetan. Bi lan hauen helburu partzialak ezberdinak izan arren, helburu nagusia komuna dute, hain zuzen ere, traza-mailako elementuen bidezko kutsaduraren azterketa Nerbioi-Ibaizabal ibaiaren itsasadarrean.

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[1] FDEZ-ORTIZ DE VALLEJUELO S. 2008. *Diagnóstico, evolución y predicción de la concentración metálica en el estuario del río Nerbioi-Ibaizabal*. Doktorego tesia. Euskal Herriko Unibertsitatea, Bilbo.

Tesi honen helburuak garbiak dira. Alde batetik, kutsadura metalikoaren denborarekiko bilakaera eta espazioarekiko aldakortasuna aztertzea itsasadarrean. Beste aldetik, Europako Uraren Zuzentarauaren gomendioei jarraituz, kutsatzaile metalikoen analisirako erreminta eraginkorragoak garatzea, bai metodo analitikoei dagokienez, zein emaitzen tratamendu eta interpretazioari dagokionez.

Xedeak erdiesteko bide desberdinak jorratu dira. Alde batetik, Nerbioi-Ibaizabal itsasadarreko uretan eta sedimentuetan zaintzarako monitorizazio-saio periodikoak (3 hilabetero) aurrera eraman dira. Bildutako laginetan traza-mailako elementuen kontzentrazioa determinatzeaz aparte, beste zenbait propietate fisiko-kimiko ere neurtu dira. Nerbioi-Ibaizabal itsasadarraren egungo egoera ekologikoa ikertu ahal izan dugu honela, sei urtetan zehar bilduriko datuak (2005eko urtarriletik 2010ko urrira doazenak) era desberdinetan landuz: i) espazioarekiko aldakortasuna eta denborarekiko bilakaera aztertzeko teknika estatistikoak eta grafikoak erabiliz; ii) metalen ahalmen kutsatzailea eta toxikologikoa estimatzeko indize desberdinak aplikatuz eta iii) joera nagusien identifikaziorako teknika kimiometrikoak erabiliz. Bestetik, kimika berdearen printzipioetan oinarrituz, eta gaurko metodologiarik ohikoenak garestiak, luzeak, eta ingurugirorako zein gizakirako arriskutsuak izan daitezkeela kontutan hartuz, traza-mailako elementuen determinaziorako metodo analitiko alternatiboak ere garatu dira, teknika ez-suntsikorretan eta aldagai anitzeko kalibrazio-metodotan oinarrituta. Monitorizazio-saioetatik lortzen diren aldagai anitzeko datu-sorta luzeak modu eraginkorrago batean aztertu ahal izateko teknika kimiometrikoen aplikazioa ere landu da.

Lortutako emaitzen aurkezpena ondorengo antolamenduaren arabera egin da: lehenengo eta behin **sarrera orokorra** prestatu da. Gaur egun indarrean dagoen Europako Uraren Zuzentarauari buruzko informazioa ematen da han, besteak beste, bere xedeak, mugak eta aplikatzeko orduan aurkitu diren arazoak. Zuzentarauaren aplikazioan Europako hainbat Herritan (Euskal Herrian barne) noraino heldu den ere eztabaidatzen da sarrera honetan. Ondoren, lana **hiru atal** nagusitan banatu da:

i) **Kutsadura metalikoaren monitorizazioa**. Nerbioi-Ibaizabal itsasadarrari buruzko deskribapen fisikoa eta historikoa biltzen dituen sarrera batekin hasten da atal hau. Ondoren, uren eta sedimentuen epe luzerako monitorizazio-saioak deskribatzen dituzten bi azpi-atal ageri dira. Lehenengoan uretan lortutako emaitzak laburbiltzen dira eta bigarrenean, berriz, sedimentuekin lortutakoak. Kutsaduraren denborarekiko bilakaera ikertzea da helburu nagusia bietan. Lehen atala 2009ko eta 2010ko urtarriletan buruturiko sedimentuen makrolaginketen gorabeherak azaltzen dituen 3. azpi-atalarekin bukatzen da. Makrolaginketa bakoitzean sedimentuak hartu ziren itsasadarraren 49 puntu desberdinetan, kutsaduraren distribuzio espaziala zehaztasun handiagoz aztertzea ahalbideratu zuena.

ii) **Multzoen identifikaziorako teknika kimiometrikoen aplikazioa**. Kimiometriaren alor garrantzitsua den multzoen identifikazioan erabiltzen diren hainbat tekniken oinarria birpasatzen duen sarrera batek zabaltzen du atal hau. Gero, azpi-atal bakarrean, Nerbioi-Ibaizabal itsasadarreko sedimentuetan neurtutako metalen kontzentrazioak batzen dituen datu-matrizearen analisiaren emaitzak laburbildu dira. Analisia PCA, MCR, PARAFAC eta Tucker3 multzoen identifikaziorako teknika kimiometrikoen bidez egin da, eta laginak antzeko ezaugarrien arabera multzokatzea ahalbideratu du.

iii) **Sedimentuen analisia teknika ez-suntsikorren bidez, traza-elementuen kontzentrazioak determinatzeko analisi-metodo alternatiboen garapena**. Aldagai anitzeko kalibraziorako teknika kimiometrikoen oinarriak, alde batetik, eta teknika analitiko ez-suntsikorren ezaugarriak, beste aldetik, biltzen dituen sarrera batekin hasten da 3. atala. Ondoren, bi azpi-ataletan, hurrenez hurren, X-izpien bidezko fluoreszentzian eta espektroskopia bibrazionalean oinarritutako iragarpenerako ereduak deskribatzen dira. Sedimentuetan hainbat elementuren aldi bereko analisia egiteko alternatiba moduan planteatzen dira eredu hauek. Laginaren analisi zuzena ahalbideratzen dute, laginaren tratamendu klasikoa (erauzketa) saihestuz, eta hauen garapenean aldagai anitzeko kalibrazio-teknikak erabiltzen dira.

Lanari bukaera emateko atal desberdinetan lortutako **ondorioak** bildu dira. Hauek hitzaurre honetan planteatutako helburuei erantzuna emateko saio bat dira. Ikusiko denez, kasu gehienetan asmatu bada ere, lanaren garapenean galdera berriak sortu eta bide tentagarriak zabaldu dira. Etorkizunerako beste lan baterako abiapuntu akaso. Gure gaixoak askoz itxura hobea badu ere, asko falta da oraindik inoiz erakutsi zuen indarra eta bizipoza erabat berreskuratu arte.

Ez dut hitzaurre hau bukatu nahi bere laguntza ekonomikoarekin lan hau posible egin duten erakundeei eskerrak eman gabe. Alde batetik, Euskal Herriko Unibertsitatearen ikerketa-errektoreordeari, egile honi emandako doktorego-aurreko bekagatik. Beste aldetik, Eusko Jaurlaritzari eta EHUko Unesco Katedrari, hurrenez hurren, BERRILUR II eta III (Kodeak: IE06-0179 eta IE09-242) eta OKAMET (Kodea:UNESCO 09/23) ikerketa-proiektuak onartu eta finantzatzeagatik.

Sarrera

Europako Uraren Zuzentarauaren helburuak, egokitasuna eta ezarpena: Europatik Euskal Herrira

Gredilla A.; Fdez-Ortiz de Vallejuelo S.; Arana, G.; de Diego A.; Madariaga J.M. 2011.
Ekaia, Euskal Herriko Unibertsitateko zientzi eta teknologi aldizkarian onartua.

“Ura ez da beste ondasun komertzialak bezalakoa, babestu, defendatu eta behar bezala maneiatu behar den ondarea da”. Baieztapen honetan oinarrituz, 1988. urtean, Europako kontseiluak ur-masen egoera ekologikoa hobetzeko gida edo legedien eraketa eskatu zion Europar Batzordeari Frankfurten ospatu zen ministerioen mintegian. Ordurako bazeuden 30 araudi desberdin eratuta uraren kalitatearekin erlazionatuta (nekazaritzak sorturiko kutsaduraren aurkako legedia, Uraren Garbiketarako Zuzentaraua, Hegaztien Zuzentaraua edo Habitataren Legedia, besteak beste), baina hauen gabezietan eta eraginkortasun mugatuan oinarrituz, estatu burukideak araudi guztiak bilduko zituen Zuzentarau homogeneoaren eraketa aintzakotzat hartzen hasi ziren [1]. Honekin batera, 1995ean Europako ingurugiroaren agentziak urak kuantitatiboki eta kualitatiboki babesteko beharra baieztatzen zuen informea aurkeztu zuen. 1999an aurreko eskakizun guztiak Europako parlamentuan aurkeztu ziren eta, azkenik, 2000. urtean Europako Uraren Zuzentaraua (*European Water Framework Directive, EWFD*) moduan ezagutzen dena onartu eta eratu zen [2]. Legedi honek une horretara arte Europa mailan, urari dagokionez, ezagutzen zen egitaraurik zehatzena eta zabalena ekarri zuen [3]. Honen arabera 2015. urterako Europar Batasuneko Estatu Kide guztiek Zuzentaraua ezarri eta beraien urak (barnealdeko urak, trantsizio-urak, kostaldeko eta lurpeko urak barne) “egoera ekologiko ona” dutela (*Good Ecological Status*, GES) ziurtatu behar dute [4]. Zuzentarauak gainazaleko eta lurpeko urei egiten die erreferentzia, nahiz eta askotan gainazaleko urei buruz bakarrik hitz egiten den. Helburu orokor hau erdiesteko, kostu osoaren berreskurapenerako legedia garatu zen (*Full Cost Recovery*, FCR). Honek uren finantza kostua (saneamendurako kostuak), uren baliabide ekonomikoak (uraren aldizkako erabilera edo erabilera desegoki baten ondorioz sortutako gastuak) eta ingurugiroko kostuak (giza ekintzen ondorioz gertatutako uraren alterazio fisiko eta biologikoek eragindako gastuak) barneratzen ditu. Behin “uraren kostua” zehazki kalkulatu denean, hau da, uraren kalitate ekologiko ona eskuratzeko beharrezkoak diren gastu ekonomikoak zehaztu direnean, kostuak uren erabiltzaileen artean banatu behar dira Zuzentarauaren 9. Artikuluan zehazten den moduan [5]. Teorikoki, gastu hauek guztiak irabazi bihurtuko lirateke, WFD-ren ezarpenak ekarriko dituen hobekuntzen ondorioz. Europako Ingurumenaren Batzordearen arabera irabaziak hurrengo hiru talde nagusitan banatuko lirateke [3]: i) irabazi komertzialak: ur garbiak tratamendurako gastu gutxiago beharko luke, beraz, galerak arlo honetan minimoak izango lirateke; ii) erabiltzaileen irabaziak: aisiarako zuzendutako aktibitateek sortuko luketena eta iii) ez-erabiltzaileen irabaziak: ur guztiak egoera ekologiko ona lortu dutela jakiteak emango lukeen irabazi pertsonala.

1. Europako Uraren Zuzentarauaren helburu orokorrak

Aurretik aipatu den bezala, Zuzentarauak ur guztien egoera ekologiko ona lortzea finkatu zuen helburu orokor moduan eta, aldi berean, baita larregi eraldaturiko uren identifikazioa (*Heavily Modified Water Bodies*, HMWB) burutzea ere. Baina zertan datza aipatutako egoera ekologiko ona? Ur-masa batek estatu ekologiko ona lortzen du uraren kalitate biologikoa ziurtatzen dituzten parametro adierazgarriek (fisiko-kimikoak, hidromorfologikoak eta biologikoak) giza-jarduerek eragindako alterazio baxuak aurkezten dituztenean. Hau da, beraien balioak inolako eraldaketarik jasan ez duen uraren balioetatik gutxi urruntzen direnean erdiesten da egoera ekologiko ona.

Asmo nagusi hauetaz aparte, beste bi helburu nagusi finkatu zituen Zuzendaritzak: substantzia kaltegarrien ezabatze osoa edo etengabeko murriztea eta kostaldeko uretan, jatorri naturaleko substantziei dagokionez, balio naturaletik hurbil dauden kontzentrazioak eskuratzea, eta substantzia artifizialei dagokienez, berriz, zero inguruko balioak lortzea. Hauekin batera helburu partzial batzuk ere definitu ziren: i) ur-baliabideen egoera hobetzea; ii) uraren erabilera iraunkorra zabaltzea; iii) lurpeko uren kutsadura murriztea eta kontaminazio berriak saihestea eta iv) uholde eta lehorteen efektuak murriztea.

2. Zuzentaraua aplikatzeko eta betetzeko jarraibideak

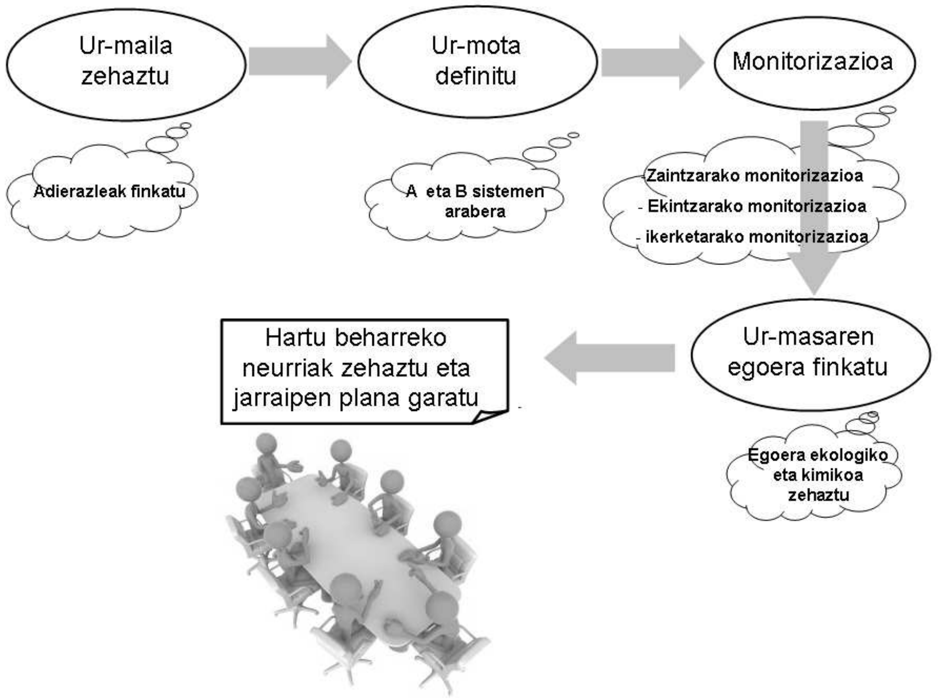
Argi dago Europako Uraren Zuzentarauaren aplikazioa prozesu luzea eta zaila dela, epe laburrean aldatu eta aplikatu beharreko arauak asko baitira.

Jakina da gainera, aztergai diren urak oso aldakorrak direla, eta honen ondorioz beraien azterketa nahiko konplexua izaten dela. Aipatutako aldakortasunak uren propietate fisiko-kimikoetan gertatzen diren bat-bateko aldaketen ondorioz sortzen diren denborako eta espazioko aldaketetan du jatorria. Aldaketa espazialen eragile nagusia ur-masen heterogenotasuna da. Uretan gertatzen diren aldaketa hidromorfologikoek, kutsadura puntual edota difusoek eta prozesu biologikoek eta kimikoek ere parte hartu dezakete aniztasun espazial horretan. Denboran gertatutako aldaketak, aldiz, urte-sasoiaren arabera gertatzen diren prozesu biologikoen edota aldaketa hidrologikoen eta hidrometeorologikoen (elurteen, uholdeen, eta abarren) ondorioz gertatzen dira batez ere [6]. Batzordeak ur-masa guztien ikerketa sakona eskatzen duenez, aldaketa hauek guztiak kontuan hartu beharrekoak dira. Zuzentarauaren esanetara gainera, Estatu Kide bakoitzak bere mugetan dauden ur-masa bakoitzerako jarduera plan bereizgarria eta zabala diseinatu behar du. Helburu horrekin ur-masa bakoitza sakonki aztertu behar da, haren ezaugarriak ikertu, haietan gertatutako giza-eraginak baloratu eta ur bakoitzaren erabileraren analisi ekonomikoa egin.

Horrekin erlazionatuta, WDF-aren 2. eta 5. eranskinetan ur-masen azterketa sakona burutzeko jarraitu beharreko hurrengo urrats nagusiak azaltzen dira; i) aztergai den ur-masa ezaugarritzea; ii) uraren egoera ekologikoaren eta kimikoaren azterketa eta iii) plangintza-hidrologikoaren eraketa. 1. Irudian Zuzentarauaren aplikaziorako jarraitu beharreko urratsen eskema ikus daiteke. Jarraian urratsak banan-banan aztertuko dira.

2.1. Ur-masaren ezaugarritzea

Aztergai den gainazaleko ur-masa ezaugarritzeko lehenengo urratsean ur-masa ondoko kategorien artean sailkatu behar da: ibaia, aintzira, kostaldeko ura, trantsizio-ura, eta gainazaleko ur artifiziala edo oso eraldatua. Ur-kategoria zehazki definitzea ezinbestekoa da aurrerago haren egoera ekologiko eta kimikoa era egokian definitu ahal izateko, kategoria bakoitzak parametro adierazle batzuk baititu aztertzeko. Parametro horiek hiru talde nagusitan bana daitezke: adierazle biologikoak (fitoplanktona eta algak, besteak beste), hidromorfologikoak (mareak, ezaugarri morfologikoak, eta abar) eta fisiko-kimikoak (tenperatura eta disolbaturiko oxigenoaren kantitatea, adibidez).



1. Irudia. Zuzentarauaren ezarpenerako eskema orokorra

Horietako batzuk komunak dira ur-kategoria guztietarako eta beste batzuk espezifikoak kategoria bakoitzean [7].

Behin aztergai den ur-masaren kategoria definitu denean, ur-masa zein motakoa den zehaztu behar da. Honetarako Zuzentarauak sailkapenerako bi sistema proposatzen ditu, A eta B sistemak. A sistemaren arabera urak, lehenik, kokapen geografikoaren arabera sailkatu behar dira, horrela ur-masaren inguru ekologikoa edo eko-eskualdea mugatu ahal izateko. Arroaren kokapen geografikoari dagokionez Europar Batasuneko urak 6 inguru ekologiko nagusitan bana daitezke: i) Ipar Atlantiko ozeanoa, ii) Itsas Baltikoa, iii) Barentseko Itsasoa, iv) Norbeiako Itsasoa, v) Iparraldeko Itsasoa eta vi) Mediterranear Itsasoa. Ondoren, inguru ekologiko bakoitzeko ur-masak ur-mota ezberdinetan banatu behar dira beraien tipologiaren arabera.

B sistemaren arabera, aldiz, ur-masen sailkapenerako adierazgarriak diren zenbait parametro fisiko-kimiko hartu behar dira kontuan. Zuzentarauak derrigorrez kontuan hartu beharreko parametro batzuk finkatzen ditu: latitudea, longitudea, mareen eragina eta uraren gazitasuna, besteak beste. Aukerako beste parametro adierazgarri batzuk ere definitzen ditu: trantsizio-uretan, adibidez, itsasgoraren eta itsasbeheraren altuera eta uraren erresidentzia-denbora kontuan hartzea gomendatzen da. Kostaldeko uretan, aldiz, olatuen altuera aztertzea proposatzen da beste faktore batzuen artean [8].

Adibide moduan Zuzentarauak trantsizio-uren kategoriarako definituriko A eta B sistemak ikus daitezke 1. eta 2. Tauletan.

2.2. Egoera ekologiko eta kimikoaren azterketa

Orain arte monitorizazioa izan da gainazaleko ur-masa baten egoera ekologiko eta kimikoa ezagutzeko bide ohikoena, aztergai den zonalde bakoitzean. Hau da WFD-ak gomendatzen duena. Errealitatean Zuzentarauan ez dira espezifikazio ugari ematen jarraitu beharreko monitorizazio saioei buruz (laginketa-saioen maiztasuna edota zehazki aztertu beharreko parametroak, adibidez, ez dira zehazten).

1. Taula. Trantsizio-uren kategoriarako WFD-ak proposaturiko A sistema.

A sistema	**Deskribapena**
Eko-lurraldea	Ipar Atlantiko ozeanoa Itsas Baltikoa Barentseko Itsasoa Norbeiako Itsasoa Iparraldeko Itsasoa Mediterranear Itsasoa
Mota	- Urteko batazbesteko gazitasunean oinarrituta: < ‰ 0.5: ur geza ‰ 0.5 - 5: oligohalinoa ‰ 5 - 18: mesohalinoa ‰ 18 - 30: polihalinoa ‰ 30 – 40: euhalinoa - Mareen batazbesteko anplitudean oinarrituta: < 2m: mikromareala 2 – 4m: mesomareala > 4m: makromareala

2. Taula. Trantsizio-uren kategoriarako WFD-ak proposaturiko B sistema.

B sistema	**Deskribapena**
Derrigorrezko ezaugarriak	Latitudea Longitudea Mareen anplitudea Gazitasuna
Aukerako ezaugarriak	Sakonera Ur-lasterraren abiadura Olatuetarako esposizio-denbora Uren erresidentzia-denbora Uren batezbesteko tenperatura Ur-nahastearen ezaugarriak Uhertasuna Substratuaren konposizioa Itxura fisikoa Uraren tenperatura aldaketak

Zuzentarauaren 8. artikuluan uraren egoera aztertzeko laginketa-programa egokia eta sakona egiteko beharra besterik ez da aipatzen. Hala ere, hiru mailako monitorizazio-sistema eskatzen da 5. eranskinean. Laginketa programa egokiak ondoko hiru monitorizazio-maila hauek hartuko lituzke barnean: i) zaintzarako monitorizazioa, ii) ekintzarako monitorizazioa eta iii) ikerketarako monitorizazioa [2]. Lehenengoa giza-aktibitateak eragindako epe luzerako alterazioak aztertu nahi direnean eraman behar da aurrera; bigarrena aztergai den ur-masak Zuzentaraua ez betetzeko arriskua duenean aplikatu behar da eta hirugarrena kasu berezietan bakarrik erabili behar da (aldizkako kutsadura baten eraginak aztertu nahi direnean edota zonalderako ezarritako muga-balioak gainditzearen arrazoiak ezagutzen ez direnean) [9].

Aipaturiko hiru mailako monitorizaziorako eskema 2006. urterako ezarrita egon behar zen arren, haren aplikazioak Europa mailan zenbait oztopo izan ditu; alde batetik, aurrera eramandako programa gehienek ez dituzte WFD-ak ezarritako baldintzak bete eta, bestetik, uren kalitatea aztertzeko monitorizatu beharreko parametro adierazgarriak zehazki finkatu ez direnez, lortutako datuak, kasu askotan, ez dira konparagarriak [10].

Jakina denez, monitorizazio-programa baten baliogarritasuna eskuratzen dituen datuen kalitatean oinarritzen da, hau da, bilduriko datuak guztiz fidagarriak eta konparagarriak izan behar dira. Esan daiteke monitorizazio-saioa guztiz egokia dela datu eskuragarri, fidagarri eta beste datuekin konparagarriak sortzen dituenean. Hau lortzeko eta laginketa-arloan gertatu diren hainbat hutsegite konpontzeko asmoz, Europa mailan zenbait talde aritu dira lanean; 2001ean, esaterako, CIS (*Common Implementation Strategy*) eratu zen. Lantalde honek lurpeko uren laginketa egokia garatzeko zenbait gida eratu ditu [11]. Trantsizioko eta kostaldeko uren ikerketari dagokionez bestetik, COAST izeneko taldea aritu da beste talde batzuen artean [8]. Hala ere, talde hauek guztiek laguntzarako gidak besterik ez dituzte garatu, izaera legalik ez baitute.

Gaur egun, Europa mailan laginketa-eredu ohikoena unez-uneko ur-bilketan oinarritzen da, “bottle sampling” moduan ezagutzen dena. Eta hau da WFD-ak definituriko hiru laginketa-mailetan askotan erabilia izan dena.

Logikoa denez, metodo honen bitartez bilduriko ura ezin da lagin adierazgarri moduan definitu, une zehatz bateko informazioa bakarrik ematen baitu. Gainera, aurretik aipaturiko espazio- eta denbora- aldaketek edota bat-bateko eragin industrial batek erabat muga ditzakete gainazaleko uren propietateak. Honi monitorizazio-saio batek ekartzen duen kostu ekonomikoa gehitu behar zaio. Laginketa-eredu honen gabeziak konpontzeko asmoz hainbat teknika garatu dira azken urteotan, esate baterako, kontrol urruneko aginduetan oinarritutako monitorizazio-sistemak [12], ur-emarien diseinuetan oinarritutako azterketak, degradazio-koefizienteen kalkuluen bitartez buruturiko analisiak [13-15] edo eratu diren hainbat eredu matematiko: HSPF (*Hydrological Simulation Program- FORTRAN*) [16], AGNPS (*Agricultural Non-Point Source Pollution Model*) [17] eta SWIM (*Soil and Water Integrated Model*) [18], beste batzuen artean.

Aztergai den ur-masaren sailkapena burutzeko beharrezko informazio guztia bildu denean, bere egoera (“status” moduan ezagutzen dena) zehaztu behar da. Honetarako “Environmental Quality Ratio” (EQR) balioak kalkulatu behar dira. EQR koefizienteak ur-masa horretarako definituriko parametro adierazgarrien balio esperimentalak zonalderako definituriko erreferentzia-balioekin (*Ecological Quality Standards*, EQS) zatituz lortzen dira. Eskuratzen diren koefizienteak 0 eta 1 tartean daude: 0tik hurbileko EQR-ek egoera edo status txarra adieraziko lukete eta 1etik hurbileko balioek, aldiz, oso egoera ona [19]. Gero ikusiko denez, alabaina, erreferentzia-balioen definizioa ez da batere erraza.

EQR-ak adierazgarriak diren parametro biologikoetarako, hidromorfologikoetarako eta fisiko-kimikoetarako soilik kalkulatuz gero, uraren egoera ekologikoa definitzen da. Oso egoera onetik egoera oso eraldatura doazen bost mailako sailkapena egin daiteke lorturiko koefizientearen arabera. Osagai kimikoetan oinarrituz gero, aldiz, ur-masa bi mailatan sailka daiteke bakarrik, bere egoera kimikoa zehaztuz. Egoera ekologikoak eta kimikoak ur-masaren benetako egoera edo aipaturiko “status”a definitzen dute (ikusi 3. Taula) [20].

Ur-masen sailkapen egokia burutzea ezinbestekoa suertatzen da, honen arabera mugatzen baitira gerora hartu beharreko neurriak. Adibidez, egoera ona duen ur-masa maila moderatuan sailkatuko balitz hobekuntza plan bat garatu beharko litzateke, baina horrek beharrezkoa ez den kostu ekonomikoa ekarriko luke. Alderantziz, maila moderatua duen ura ez dagokion maila goreneko batean finkatuko bagenu, ez litzateke hobekuntza planik garatuko eta horrek ez luke une horretan gasturik sortuko, baina biharko egunean gastu handiagoa ekarriko luke [6].

3. Taula. Ur-masen sailkapenerako WFD-ak proposatzen duen eredua.

Aztergai diren parametroak	**Maila-kopurua**	**Sailkapena**		
Osagai biologikoak	5	Egoera biologikoa	***Egoera Ekologikoa***	**Ur-masaren egoera**
Propietate Hidromorfolog.	5	Egoera hidromorfolog.		
Propietate fisiko-kimikoak	5	Egoera fisiko-kimikoa		
Elementu kimikoak	2		***Egoera Kimikoa***	

Baina sailkapen egokia eta zehatza aurrera eramateko erreferentzia-balioen (EQS-en) definizio egokia eta doia egitea ezinbestekoa da. “*Ur-kategoria bakoitzerako oso egoera ekologiko onerako definituriko baldintza biologikoak, hidromorfologikoak eta fisiko-kimikoak definituko dira erreferentzia-balio bezala”*. Hau da WFD-ak bere 5. eranskinean zehazten duena. Erreferentzia-balio horiek lortzeko zenbait bide proposatzen ditu batzordeak: i) eraldaketarik ez edo oso eraldaketa txikia izan duten espazioetan oinarritzea; ii) zonaldearen datu historikoak eskuragarri izatea edo iii) espezialisten aholkua izatea.

Aipatzen diren bideak ez dira gehienetan oso praktikoak izaten. Kasu gehienetan lehenengo aukera ez da posiblea izaten, Europa mailan eraldaketarik izan ez dituzten zonaldeak ez baitira erraz aurkitzen. Bigarrena ere nahiko ez-ohikoa izaten da, normalean eraldaketak gertatu ondoren hasten baitira biltzen datuak. Zenbait kasutan zonalde birtualak eratu dira erreferentzia-balio posibleekin [21], baina arlo honetan garatu beharreko bidea luzea da oraindik.

2.3. Plangintza-hidrologikoaren eraketa

Aztergai den ur-masaren informazioa bildu denean, Estatu Kideek arroaren plangintza-hidrologikoa garatu behar dute. Honetan, Zuzentarauaren 7. eranskinean aipaturiko datu guztiak azaldu behar dira, esaterako:

1) Mugape hidrologikoaren deskribapen orokorra, ur-masaren mapa eta mugak argi zehaztuz eta erreferentzia-baldintzak definituz

2) Ur-masaren gaineko giza-ekintzak eta hauek eragindako eraldaketak

3) Babesturiko zonaldeen identifikazioa

4) Monitorizaziorako erabili diren puntuen kokapena azaltzen duen mapa eta lorturiko emaitzak (mapan bertan azalduz)

5) Finkaturiko ingumeneko helburuak zehazten dituen zerrenda

6) Uraren analisi ekonomikoaren laburpena, berreskurapenerako gastuak azalduz

7) Zuzentarauaren ezarpenerako garatu diren plangintzen laburpena

8) Ur-masa txikiagoetarako (normalean beste ur-masa handiagoen parte direnak) garaturiko plangintza berezien erregistroa

9) Jendaurrean aurkeztuko den informazioaren laburpena

10) Erabakietan parte hartuko duten autoritateen zerrenda.

3. Zuzentarauaren egokitasuna: eztabaida kritikoa

3.1. Analizatu beharreko konpartimentuak

Europako Uraren Zuzentarauan argi aurkezten dira uretan aplikatu behar diren neurriak ur-masa guztien egoera ekologiko egokia eskuratu nahi bada, baina aipamenak besterik ez dira egiten sedimentu eta biotan hartu beharreko neurriei buruz. Bi kontzeptu horiek gutxitan aipatzen dira idazkian zehar [22], adibidez: 16. artikuluan "*Batzordeak kalitatezko estandarrak (EQS-ak) proposatu behar ditu lehentasuna duten substantzietarako* (lehentasuna dutela esaten da duten gaitasun kutsatzailea dela eta)*, uretan, sedimentuetan eta biotan*" aipatzen da, eta 2. artikuluan, "*EQS-ak, uretan, sedimentuetan eta biotan gainditu behar ez diren osagai kutsatzaileen mugako kontzentrazioak"* direla azaltzen da. Hauetaz aparte ez da bestelako argibiderik ematen gainazaleko uretan funtsezko papera duten sedimentu eta biotari buruz [23]. Kontraetsenplu moduan, araudi osoan zehar uraren analisiaren garrantzia momentu oro azpimarratzen da, eta "ur" hitza 400 bat aldiz errepikatzen da idazkian zehar. Hori dela eta, orain arte EQS balioak uraren kasurako bakarrik garatu dira [24]. Hala ere, WFD-ak gainazaleko uren monitorizazio sakona eskatzen du bere egoera ekologikoa eta kimikoa ondo definitu ahal izateko. Honek gainazaleko uretan eragingarriak diren konpartimenduak analizatzea eskatzen du. Gutxienez ura, biota eta sedimentuaren azterketa sakona burutu beharko litzateke aztergai den ur-masari buruzko informazio osoa lortzeko (batez ere trantsizioko eta kostaldeko ur-masei dagokienez). Itsasadarretan sedimentua eta biota sakonki analizatu dira, informazio garrantzitsua ematen baitute kutsatzaile espezifiko batzuei buruz [25-30].

Batzordearen oinarrietako bat "bat kanpoan, denak kanpoan" baieztapenean datza (*one out, all out*), hau da, monitorizaziorako lehentasuna duten konposatuen artean egoera txarrena duenak finkatuko du ur-masa osoaren egoera. Lelo honetan oinarrituz, ur-masen ikerketa sakona (ura, sedimentua eta biota barne) inplizituki eskatzen da [31].

Sedimentuek eragindako kontaminazioa mundu-mailako arazoa da, batez ere iragan industriala izan duten inguruetarako. Kostaldean eta trantsizio-uretan giza-jarduerek eragindako eraginen adierazle izan dira beti, denbora luzez kutsatzaile organikoak zein ez-organikoak pilatzeko gai baitira. Normalean sedimentuetan biltegiratutako konposatuek ez dute organismo bizietarako arriskua ekartzen, eskuragarri ez baitaude [32, 33]. Hala ere, ur-masa hauek inguru natural oso aldakorrak direnez (besteak beste, bat-bateko pH eta gazitasun gradiente horizontalak eta bertikalak gertatzen dira uretan) konposatu kutsatzaileak berdisolba daitezke ur-fasera itzuliz eta, ondorioz, organismoetarako eskuragarri bilakatuz [34-36].

Sedimentuen kontrola eta azterketa mundu osoan landu izan da. Ipar Ameriketan laster eratu eta ezarri ziren sedimentuen egoera kontrolatzeko lehenengo arauak. Europa mailan, aldiz, sedimentuen ikerketarako garatu diren bideak, laginketa saioak eta gidak, besteak beste, nahiko desberdinak izan dira beraien artean eta 2000. urtean WFD-aren ezarpenaren ondoren aipatutako dispertsioa ez da gehiegi aldatu.

Bestalde, Zuzentarauan zaintzarako monitorizazioa aipatzen denean ez da zehazten adierazgarriak diren parametro fisiko-kimikoak zein espazio fisikotan aztertu behar diren. Argi azaltzen da, aurretik aipatu den moduan, bi eratako parametro kimikoak daudela: egoera ekologikoa baldintzatzen dutenak (ezaugarri fisiko-kimikoak alegia) eta egoera kimikoa bera zehazten dutenak. Baina egoera kimikoa uretan, sedimentuetan eta biotan zehaztea ez da batere prozesu erraza. Uraren kasuan, WFD-ak aipaturiko EQS balioen garapenerako egindako ahaleginak nabariak izan dira, baina sedimentuetan eta biotaren kasuan erreferentzia-balioak ez dira oraindik gehiegi landu. Hauen gabezia dela eta, konparaketarako bide desberdinak erabili izan dira sedimentuen eta biotaren egoera ekologiko eta kimikoa aztertzerako orduan, hots:

- Aztergai den inguruaren egoera kimikoa finkatuko dituzten parametro adierazgarrien (elementu metalikoen eta konposatu organikoen edukia, adibidez) balio naturalak ezagutzen badira, hauek erabili izan dira maiz erreferentzia-balio moduan. Honen adibide gisa mundu-mailan sedimentuen azterketarako askotan aplikatu izan diren geoakumulazio-indizeak ditugu [37].
- Inguruaren balio naturalei buruzko informazio zehatza eskuragarri ez dagoenenan antzeko propietateak dituen sedimentuaren edota biotaren balioak erabili izan dira zenbait kasutan erreferentzia moduan [22].
- Zuzenean edo zeharka erlazionaturiko legediak ere erabili dira konparaziorako. Esaterako, sedimentuetan pilaturiko elementu kimikoen eragin biologikoa aztertzen duten SQG-ak (*Sediment Quality Guidelines*) sarritan aplikatu izan dira. SQG mota desberdinak garatu dira, esate baterako, ERL (*Effects Range-Low*), ERM (*Effects Range-Median*), AET (*Apparent-Effects Threshold*) eta PEL (*Probable-Effects Level*) [38-43].

3.2. Analizatu beharreko elementuak

Batzordeak sedimentu eta biotaren azterketaren beharra gomendatu ez izana akats argia da, baina nahikoa da konpartimendu ezberdinetan aztertu eta analizatu beharreko parametroei buruz emandako informazioa?

Jadanik komentatu denez, ur-masa baten egoera zehazteko beharrezkoak diren parametro adierazgarrien monitorizazioa nahitaezkoa da. Adierazgarriak diren parametro biologikoengan eragina duten parametro hidromorfologikoak eta fisiko-kimikoak luze aipatzen dira Zuzentarauaren 5. eranskinean, baina parametro kimikoei dagokionez ez da zehaztasun handirik ematen, soilik lehentasuna duten substantziak aipatzen dira. Lehentasuna aitortzen zaie duten gaitasun kutsatzailea dela eta. 8. eranskinean kutsatzaile nagusi hauei buruzko zerrenda hedatua aurki daiteke. Besteak beste, ondorengo konposatuak aipatzen dira: konposatu halogenatuak, fosforodun konposatu organikoak, konposatu organoeztanikoak, hidrokarburo iraunkorrak, metalak eta beraien konposatuak, eta artsenikoa eta bere konposatuak, beste batzuen artean. Zerrendan dauden konposatu gehienak kutsatzaile organikoak dira eta metalak modu orokorrean besterik ez dira aipatzen, tributileztainuarekin batera. Kutsakortasun handiko beste elementu eta konposatu asko ez dira aipatu ere egiten Zuzentarauaren idazkian zehar.

Jakina da kutsatzaile kimikoen artean garrantzitsuenetarikoen artean PAH-ak (*Hidrokarburo Polizikliko Aromatikoak*), PCB-ak (*Bifenilo Polikloratuak*, konposatu halogenatuen barnean sartzen direnak) eta zenbait metal eta metaloide (As, Cd, Cr, Cu, Hg, Ni, Pb, Sn eta Zn) daudela. Guztiak kutsatzaile garrantzitsu moduan ezagutzen dira bizidunetan pilatzeko duten gaitasuna dela eta [44]. Metalek itsasadarretan, esaterako, nahiz eta kontzentrazio oso baxuetan agertu, toxikotasun maila handia erakusten dute, batez ere beraien iraunkortasunagatik, sedimentuetan denbora luzez egoteko gaitasuna baitute [45].

Beraz, WFD-ak proposaturiko hiru mailako monitorizazio-programa egokia garatzeko analizatu beharreko konpartimenduak (ura, sedimentua edota biota) eta hauetan neurtu beharreko parametro adierazgarriei buruzko informazioa osatzea beharrezkoa litzateke Zuzentarauaren eskutik, sarritan aipatzen diren datu konparagarri eta fidagarriak eskuratu ahal izateko.

3.3. Analisirako metodoak

Europar batzordearen partaide den AMPS (*Analysis and Monitoring of Priority Substances*) talde espezializatuak, Estatu Kide guztiek eskuraturiko datuen konparagarritasuna eta fidagarritasuna lortzeko, ziurgabetasun hau eskatzen du: EQS-aren %30eko kontzentraziorako %25eko ziurgabetasun bateratua (*combined standard uncertainty*, erroreen hedapenaren bidez kalkulatua), edota %50eko ziurgabetasun hedatua (*standard uncertainty*, neurketa errepikatuen bidez estimatua) %95eko konfiantza-mailan, uretan analizaturiko substantzia guztietarako. Hau Europako 10 laborategitan lorturiko ziurgabetasunetan oinarrituz finkatu zen. Baina, susmagarria denez, irizpide hau lehentasuna duten substantzia askoren analisian ez da oraindik eskuratu. Are gehiago, kasu askotan erreferentzia-materialak falta dira lehentasuna duten zenbait substantziaren analisien zehaztasuna eta ziurgabetasuna determinatzeko.

Bestetik, Estandarizaziorako Nazioarteko Erakundeak (*International Organization for Standarization*, ISO) uretan egindako analisien detekzio-muga adierazgarri den mugako balioaren (EQS-aren, adibidez) %30ean jartzea proposatu du [46]. Ur-masa baten azterketan egin beharreko analisietan aipaturiko kalitateko parametroak (EQS-ak) eskuratzeak ondokoa eskatuko luke:

- Uretan, sedimentuan, biotan eta esekiriko material partikulatuan ere (*Suspended Particulate Matter*, SPM), hainbat elementu eta konposatu kimiko analizatzeko metodo berriak garatu beharko lirateke, ISO-k proposaturiko detekzio-mugetara heltzeko.

Zuzentarauaren arabera, lehentasuna duten metalen analisian disolbaturiko zatia bakarrik monitorizatu eta kuantifikatu behar da. Konposatu organikoen kasuan, ordea, kontzentrazio osoa (disolbatuta dagoena eta frakzio partikulatua) hartu behar da kontuan. Gainera ur/sedimentu banaketa-indizea (log K_p) ≥ 3 duten substantziak, uretan analizatu beharrean, esekiriko material partikulatuan neurtzea gomendatzen du Zuzentarauak, edota posiblea izanik, sedimentuetan eta biotan. Baina, zoritxarrez, SPM-an, sedimentuetan eta biotan konposatu kimiko hauen determinaziorako metodo estandarizaturik ez da garatu oraindik. SPM-n egindako analisietan, adibidez, orain arte erabili izan diren metodoak luzeak eta garestiak dira, eta beraien konparagarritasuna ziurtatzeke dago. Uretan disolbaturiko metalen analisirako, ordea, zenbait teknika estandar onartzen dira nazioartean: induktiboki eratutako plasma duen igorpen atomikoko espektrometria (ICP/AES), induktiboki eraturiko plasma duen masa espektrometria (ICP/MS) edota grafitozko labea duen xurgapen atomikoko espektrometria (ET-AAS), besteak beste [47]. Hala ere, ur-laginen laginketarako aurretratamendu metodoen bateratzea beharrezkoa izango litzateke; adibidez, lagina nola hartu eta biltegiratu adostu beharko litzateke.

Orokorki uren analisirako metodoak nahiko garatuak eta bateratuak diren arren, ur gaziaren kasuan oraindik ez da metalen analisirako metodo estandarrik finkatu, ur hauetan laginaren aurretratamendua arretaz zehaztu eta adostu beharreko urratsa baita.

- Erreferentzia-material berriak prestatu beharko lirateke. Erreferentzia-materialak (*Reference Material,* RM) eta, are gehiago, erreferentzia-material zertifikatuak (*Certified Reference Material*, CRM) analisien zehaztasuna eta trazabilitatea ziurtatzeko funtsezkoak dira, hots, analisien kalitatea zehazteko nahitaezkoak dira.

Erreferentzia-materialak hiru multzo nagusitan bana daitezke:
i) konposatu kimikoetarako RM-ak; ii) propietate fisiko-kimikoetarako RM-ak eta iii) ingeniaritzako materialentzako RM-ak. Ingurumeneko laginei dagokienez, lehenengo taldekoak dira normalean erabiltzen direnak [48]. Ingurumeneko CRM-en ekoizpenean eta kudeaketan BAM (*Federal Institute for Materials Research and Testing*), IRMM (*Institute for Reference Materials and Measurements*), IAEA (*International Atomic Energy Agency*), NIST (*National*

Institute for Standars and Technology), NIES (*National Institute for Environmental Studies*), NMIJ (*National Metrology Institute of Japan*) eta NRC-INMS (*National Research Council of Canada, Institute for National Measurement Standards*) bezalako erakundeak aritu izan dira. Konposatu ez-organikoei dagokienez sedimentuzko eta biotazko erreferentzia-material ugari eratu dira (NIST 1646, PACS-2, MESS-3, eta abar). Aldiz, urari dagokionez erreferentzia-material gutxi aurki daitezke, eta daudenak oso kontzentrazio baxukoak dira (SRM-1640, CASS-4, SLRA-4, eta abar). Konposatu organikoen kasuan, bestetik, eskuragarri dauden erreferentzia-materialak gutxiago dira; uren kasuan esaterako, ez da CRM-rik garatu konposatu organikoen determinaziorako [49]. Gehien bat sedimentuak (SRM-1944, LGC6188, BCR-536, eta abar) eta hondakin-lohietarako materialak (BCR-088, BCR-677 eta LGC6182, esaterako) landu dira. Beraz, nahiz eta azken urteotan CRM-en ekoizpena nabarmenki handitu den, horien presentzia ez da nahikoa lehentasuna duten elementu guztien balioak gainazaleko ur-masen konpartimendu guztietan determinatzeko burutu beharreko analisak zertifikatzeko orduan [50].

- Azken puntua laborategien arteko lanaren bateragarritasunari dagokio. Zenbait Estatu-Kidek jadanik laborategien arteko elkarlana oinarritzat hartu dute beraien analisien fidagarritasuna eta konparagarritasuna ziurtatzeko, baina oraindik hau ez da lehentasuna duten substantzia guztien analisietan aplikatu [51]. Horrekin batera elkarren arteko konparazio-ariketak antolatu eta aurrera eraman beharko lirateke jadanik garaturiko erreferentziako laborategien trazabilitatea ziurtatzeko. Are gehiago, analisien kalitatea ziurtatzeko protokoloen ezarpena ere beharrezkoa litzateke.

4. Europako Uraren Zuzentarauaren ezarpena

4.1. Europako Uraren Zuzentarauaren ezarpena Europan

Uraren Zuzentaraua Europa mailan urari buruz garatu den araudi zabalena izan arren, mundu-mailan baditu aurrekari batzuk. Estatu Batuetan, esaterako, 1972an “Clean Water Act” (CWA) legedia ezarri egin zen. WFD eta CWA-ren arteko antzekotasunak nabarmenak dira helburuei dagokienez [52] baina, logikoa denez, ezarpenean desberdintasunak nabarmenak dira.

Edozein araudi berriaren ezarpenak zailtasunak dakartza eta WFD-aren kasua ez da salbuespena. Estatu Kideen egungo egoera ez da berdina, alde batetik ez baitaude ezarpenaren urrats berean eta, bestetik, ez baitituzte arauak maila edo sakontasun berean aplikatzen. Ondorioz, Zuzentarauaren ezarpena Europa mailan aztertu eta konparatu nahi denenean zailtasun anitz sortzen dira.

Teorikoki, Estatu Kide guztiek 2003. urtearen bukaerarako beraien legediak WFD-aren esanetara egokitzea lortu behar zuten, baina helburu hori ez da erdietsi [53]. Askotan, uraren azterketarako eskualde bakoitzak aldez aurretik zeukan araudi partikularrak Zuzentarau berriaren abiaraztea erraztu ordez, saihestu edo moteldu du.

Orain arte WFD-aren arabera egin diren lanetan oinarrituz, Europako ur-masen proportzio esanguratsua larregi eraldaturiko ur-masa moduan (*Heavily modified water bodies*, HMWB) sailkatu da. Holandan, Belgikan, Txekiar eta Eslovakiar Errepubliketan esaterako, ur-masen %50a HMWB mailan sailkatu zen [52].

Turkiaren kasuan, adibidez, lan nabarmenak egin dira Zuzentarauaren abiarazteari dagokionez. Ahalegin handiak egin dira, esaterako, jadanik ezarrita zeuden Ingurumenaren araudia (1983/2872) eta uraren kutsadura kontrolatzen zuen legedia (2004/25687) WFD-era egokitzeko. Gainera, beste zenbait legegintza garatu dira helburu honekin. Turkiak, ohikoak diren arazo hauetaz aparte, badu zailtasun gehigarri bat, bertoko gainazaleko uren zati handi bat bere eskualdearen mugetatik kanpo ateratzen baita. Mugaz kanpoko ur hauen azterketarako beharrezkoa da inplikatuta dauden Kide guztien parte hartzea, Zuzentaraua era egoki batean garatzeko. Baterako lana antolatzea eta adostea konplikatua izaten da sarritan [54].

Erresuma Batuan, bestetik, Ingalaterra, Gales eta Eskoziako Ingurumenaren Babeserako Agentziek hainbat urtetan bilduriko datu-baseak erreferentzia-balio moduan erabili izan dira maiz bertako ur-masen egoera ekologikoa definitzeko [55]. Ingalaterraren ekialdean dagoen Humber itsasadarrean, esaterako, WFD-aren ezarpenerako MEMG-k (*UK Marine Environment Management Group-ek*) definituriko ingurumeneko adierazleak erabili dira. Adierazle hauek aztergai den inguruaren egoera baldintzatzen duen propietate baten parametro kuantitatibo edo kualitatiboak dira (dragaturiko sedimentuaren tona-kopurua edo paduren galera, adibidez), hau da, ez dira WFD-ak monitorizatzea eskatzen dituen parametro adierazgarri ohikoak (oxigeno-portzentaia edota pHa, adibidez). Hauen helburua inguru baten egoeraren ikasketa erraztea da. Humber itsasadarraren kasuan egoera baldintzatzen zuen adierazleen artean dragatzea finkatu zen eta, honetan oinarrituz, ur-masaren egoera ekologikoa definitu zen ohikoak diren parametro adierazgarriak kontuan hartu barik [56].

Danimarkak WFD-aren inplementaziorako jadanik garatuta zuen “Environmental Objectives Act” legedia egokitu zuen. Bertan 23 ur-arro ezberdintzen dira eta hauetatik %10a baino gutxiago ur-masa oso eraldatu moduan sailkatuta dago. Gaur egun bi administrazio desberdin ari dira lanean uraren kudeaketan: estatua eta udal administrazioak. Lehenengoa planak garatzeaz arduratzen da eta udalerri bakoitzak hauek aurrera eramaten direla ziurtatu behar du. Danimarkan uraren kalitatea baldintzatu duen faktore nagusia nekazaritza izan da. Honen hobekuntzarako lan handiak egin diren arren, 2015. urterako Europar Zuzentarauak eskatzen duen ur-masa guztien egoera ekologiko ona eskuratzea ez da posible izango. Erresuma Batuan erabilitako ingurugiroko adierazleak Danimarkan ere erabili dira zenbait gainazaleko uren egoera errazago definitzeko asmoz. Roskildeko Fjordean, esate baterako, uraren nitrogeno osoaren portzentaia erabili da bere egoera ekologikoa zehazteko [57].

Frantziaren kasua desberdina da, bertan uraren kudeaketarako lanak 1969an jarri baitziren martxan. ”Comité de bassin” delako kontseilua ministerioaren, herrialde bakoitzaren gobernuaren eta arroaren tokiko kideez dago osatuta. Hauek arro bakoitzerako WFD-rako planak garatzeaz arduratzen dira. Azkenik, bertako Uraren Agentziak (Agence de l´eau) hartzen ditu erabakiak. Frantzian ur-masen %22a HMWB moduan definitu da eta %8a AWB moduan (*Alterated Water Body*). Nekazaritzaren eragina nabarmena den lurraldeetan ere eraldaturiko ur-masen kantitatea txikia da. Ondorioz, 2015erako Frantziako ur guztiek egoera ekologiko ona eskuratuko dutela uste da. 1996an eraturiko “Rhone-Mediterranean-Corsica” (RMC) Uraren Master Plana lan hauetan aritu da [1].

Azken adibide moduan Holanda dugu. Honetan txikiagoak diren eskualdeko ur-masak lurralde bakoitzaren administrazioak aztertzen ditu eta aldiz, handiagoak diren ur-masa nazionalen ikerketaz “Rijkswaterstaat,” Uraren Kudeaketarako Agentzia, arduratzen da. Holandako ur-masa askotan egin diren eraldaketa hidrologikoak kontuan hartuz eta lur hauek Europa mailan ongarri-gehigarri ehuneko handiena dutenak direla aintzat hartuz, ur-masen % 42a HMWB moduan eta % 53a AWB moduan sailkatu da. Beraz, 2015erako ur-masa gehienek ez dute egoera ekologikoa ona eskuratuko. Holandako administrazioen arabera 2015 ez da epemuga erreala, baina 2027. urtea, aldiz, errealitatera gehiago hurbilduko litzateke [58].

Euskal Herrian ere zenbait lan egin dira WFD-aren ezarpena aurrera eramateko. Dagoeneko, Eusko Jaurlaritzaren arabera, 122 ibai, 4 aintzira, 14 trantsizio-ur, 44 akuifero eta kostaldeko 4 uren ikerketa sakona burutzen ari da WFD-aren ezarpenerako. Hala ere, egin diren ikerketa gehienak kostaldeko eta trantsizio-uretan burutu dira, hauek baitira betidanik eraldaketa gehien izan dituzten gainazaleko urak [30, 59-64]. Atlantiar ozeanoan itsasoratzen diren euskal ibaiek, besteak beste, industria-jarduerek eragindako eraldaketak izan dituzte gehien bat, eta Mediterranear itsasoan itsasoratzen direnak, aldiz, nekazaritzaren ondoriozko presioak izan dituzte. 2008an eraldaturiko ur-masa guzti hauetan WFD-aren ezarpena bideratzeko eta zuzentzeko Uraren Euskal Agentzia eratu zen. Horrek erabakiak hartzeko orduan talde desberdinen parte-hartzea proposatu zuen. Era horretan, hartu beharreko neurrietan Eusko Jaurlaritzak eta udal gobernuek parte hartzeaz aparte, bestelako talde batzuek, eskualdek eta ingurugiroaren defentsarako erakundek, esaterako, ere esku hartuko lukete. Orain arte egindako lanek Euskal Herriko ur-masen egoera nabarmenki hobetu dela ondorioztatzen duten arren, uren jatorrizko baldintzak eskuratzea ezinezko xedea dela argi uzten dute [65].

4.2. Europako Uraren Zuzentarauaren aplikazioa itsasadarren kasuan

Orokorki, kostaldeko ur-masen egoera ekologikoaren azterketa asko hazi zen 90. hamarkadatik aurrera, baina zoritxarrez trantsizio-urei dagokienez ez da aurrerakuntza handirik nabaritu. Beste gainazaleko urekin konparatuz, ur-masa mota hauetan nahiko atzeratuta dago WFD-aren ezarpena: alde batetik, trantsizio-urak ez dira batere homogeneoak eta, horregatik, WFD-aren lehenengo urratsa den tipologia finkatzea ez da batere erraza. Bestetik, erreferentzia-balioak zehaztea ere zaila suertatzen da, batez ere ur horietan etengabe gertatzen diren gradienteak (gazitasunean, pHan,…) direla eta. Azkenik, WFD-ak ez du zehaztasunik ematen hain aldakorrak diren ur horien monitorizazioari buruz. Inguru horietan konpondu beharreko hutsegiteak, beraz, asko dira oraindik [59].

Betidanik jakin izan da itsasadarrak eta kostaldeko urak oso ur-masa emankorrak direla eta horregatik historian zehar intentsiboki ustiatu izan dira. Beraien inguruan garatu diren industria eta urbanizazio-guneak direla eta, iturri sozio-ekonomiko nabarmenak izan dira eta gaur ere badira.

Industriaz aparte, beraien inguruan meatzaritza, basogintza, nekazaritza eta arrain-hazkuntza sarritan burutzen dira eta horren ondorioz beraien uren kalitate ekologikoak kalte garrantzitsuak izaten ditu, besteak beste, materia organikoaren gehikuntzak eta oxigenoaren gutxitzeak dakartzatenak [66]. Are gehiago, beraien erliebea dela eta, inguru paregabetzat hartu dira garraiorako eta ur zikinen isurbide gisan [67]. Propietate hauen guztien ondorioz, itsasadarrek konposatu kutsakorren biltegi moduan jokatzen dute maiz. Itsasotik, erreka txikietatik, atmosferatik, zein beraien uretan askotan kokaturiko araztegietatik datozen konposatu organiko zein ez-organikoak erraz pilatzen dituzte (sedimentuek ere jokatzen dute askotan kutsadura-iturri gisa). Baina aldi berean lurra eta itsasoaren arteko zonaldeak direnez, baldintza oso onuragarriak dituzte era askotako organismoak hazteko. Hori dela eta, beraien balio ekologiko, biologiko, sozial eta ekonomikoa oso handia da [68]. Itsasadarren azteketan, WFD-aren ezarpenari dagokionez, hobetzeke dauden aldeak oraindik ere asko dira. Hori dela eta, beraien zaintza eta babeserako legedi bereziak eratu dira. Europa mailan “Marine Strategy Framework Directive” (MSFD) garatu zen 2008. urtean. Honek ere baditu zenbait aurrekari mundu-mailan: “Oceans Act” Estatu Batuetan eta “National Water Act” Hego Afrikan. Horien helburu nagusia WFD-aren funtsa den uren egoera ekologiko ona kostaldeko ur-masa guztietan eskuratzen dela ziurtatzea da. Logikoa denez MSFD eta WFD-aren eskumenak kostaldean gainjarri egiten dira, baina WFD-ak kostaldetik itsas-milia batera zabaltzen den bitartean, MSFD-ak 200 itsas-milia hartzen ditu barne. Bi araudi horien desberdintasun nagusia egoera ekologikoaren definizioan dago. WFD-ak adierazgarriak diren 5 parametro biologiko, hidromorfologiko eta fisiko-kimikoen monitorizazioa eskatzen duen bitartean, MSFD-ak 11 propietate kualitatibo (biodibertsitatea, ez-bertoko espezien presentzia, eta abar) erabiltzen ditu aztergai den ur-masaren egoera definitzeko. Beraien oinarria ere izatez desberdina da: WFD-ak “bat kanpoan, denak kanpoan” leloan oinarritzen da eta MSFD-a, aldiz, ez da hain murriztaile, zonalde bakoitza hobeto definitzen dituen parametro edo propietateetan oinarritzen baita ur-masen kalitatea definitu ahal izateko. Egoera ekologiko onaren esanahia ere, ez da berdina bi legedietan: WFD-ak adierazgarriak diren parametroen egoera ekologiko ona soilik eskatzen ditu eta MSFD-ak, ordea, ekologikoki garbiak, dinamikoak, ezberdinak eta osasuntsuak diren ur-masak berreskuratu nahi ditu, beraien berezko propietateekin egungo eta geroko belaunaldietarako emankor eta sostengarriak izango direlarik [69].

4.3. Europako Uraren Zuzentarauaren aplikazioa Euskal itsasadarrei dagokienez

Dagoeneko aipatu da WFD-aren aplikaziorako lehenengo urratsa aztergai den ur-masaren kategoria zehaztea dela. Euskal Herrian gainazaleko ur-masa ugarienak kostaldean daude, eta beraien garrantzia eta ugaritasuna dela-eta hauek izan dira gehien ikasi eta aztertu direnak. Itsasoaren eragin nabarmena duten ur hauek talde mugatuagotan bana daitezke:

- Ibaiadar txikiek hornituriko itsasadarrak
- Mareen eragin nabarmena duten itsasadarrak
- Mareen eragin txikia duten itsasadarrak
- Itsaso irekian kokaturiko kostaldeko zonaldeak

Lehenengo hirurak trantsizio-uren barnean sartuko lirateke eta azkeneko taldea itsasoaren eragin zuzena pairatzen duten kostaldeko ur-masek osatuko lukete. Jadanik aipatu da Euskal Herrian gaur egun 14 trantsizio-ur eta kostaldeko 4 zonalde ezberdintzen direla [59], eta guztiak Atlantiar Iparraldeko itsasoaren Eko-eskualdean daude kokatuta.

Euskal Herriko trantsizio-uretan kalte nagusien iturriak, orokorki, industria, hirigintza eta aldaketa hidromorfologikoak izan dira. Lehenengo biek gehien bat uretara heltzen den materia organikoaren eta disolbaturiko oxigenoaren kantitateak baldintzatzen dituzte. Hirugarrenak ezponden ezartze, sedimentuen dragatze, portuen eraikitze eta bestelako aldaketen ondorioz sorturiko eraldaketa kimiko, fisiko eta hidromorfologikoak sortzen dituzte [66]. Horien aurka zenbait ekintza eraginkor aurrera eraman izan dira 90. hamarkadatik aurrera, baina aipagarrienak, industria-enpresen itxieraz aparte, ur hauetara heltzen diren hondakin-uren Garbiketarako Egitasmo Iraunkorrak dira. Euskal Herriko itsasadar eta kostaldeko uretara heltzen diren uren kontrola ahalbidetzen duen garbiketarako saio gehienak jadanik ezarrita eta osatuta daude.

Euskal-Herrian WFD-aren ezarpenerako monitorizazio-lanetan ur-masa motak definitzeko erabili diren aukerako parametro adierazgarrien artean gazitasuna, disolbaturiko oxigenoaren kantitatea, mareen eragina, uraren sakontasuna, ur-emariaren abiadura eta substratuaren egitura, besteak beste, aipatu daitezke. Lehengo biak oso esanguratsuak dira ur-masa baten egoera ekologikoa definitzeko orduan. Gazitasunak, adibidez, uretan disolbaturiko elementuen kontzentrazioa mugatzen du. Oxigenoa, bestalde, uretan bizi diren organismoetarako ezinbestekoa denez, ur-masaren kalitatearen adierazle ona da (oxigenoaren asetasunaren portzentajea %100 izateak egoera ekologiko oso ona eta onaren arteko egoera adieraziko luke; %80 ingurukoak, aldiz, egoera ona eta moderatuaren artekoa, eta %60ak egoera moderatu eta pobrearen artekoa) [66].

Europako beste lurraldeetan bezala, Euskal itsasadarretan WFD-aren ezarpenerako egin diren lanetan hainbat zailtasun sortu dira, batez ere erreferentzia-balioei dagokienez. Konparaziorako beharrezkoak diren balio hauek topatzea eta zehaztea lan neketsua da, Euskal gainazaleko ur gehienak giza ekintzen ondorioz bortizki eraldatuak izan baitira. Honi irtenbidea bilatzeko asmoz (EQR-ak kalkulatzeko orduan betez ere) Eusko Jaurlaritzak 1994 eta 2002 urteen artean 32 ur-masetan (uretan, sedimentuan eta biotan) egindako analisietan lortutako emaitzak erabili dira. Datu hauek guztiak LQM (*Littoral Water Quality Monitoring and Control Network*) gidan daude bilduta [21, 70, 71]. Konparazioetarako 32 ur-masen balio naturalak (*background values*) eta bertan emandako giza ekintzak kontuan hartu dira.

LQM-ko datuetan oinarrituta Euskal Herriko zenbait ur-masen egoera ekologikoa zehazten saiatu da [8]. Metodologia zehatza erabiliz, honako emaitzak hauek lortu dira: Euskal kostaldeko ur-masen %25.5a egoera biologiko ona edo oso ona izan arren, %7.8ak soilik eskuratzen du egoera ekologiko ona; trantsizio-urei dagokienez, ez dago egoera ekologiko ona aurkezten duen ur-masarik, gehien bat, uretan neurturiko propietate fisiko-kimikoen balio eskasen ondorioz.

Honetaz aparte hainbat lan egin dira Euskal Herriko trantziziozko uren monitorizazioan eta azterketan, batez ere *Nerbioi-Ibaizabal* ibaiaren itsasadarrean eta *Oka* ibaiaren itsasadarrean [62, 72-82]. Datu ekonomiko moduan, Batzordeak Europa mailako ur-masa guztien monitorizaziorako 730 M€ beharko zirela kalkulatu zuen 1993an, eta Eusko Jaurlaritzak 2004-2005 urte bitartean egindako 20 ur-masen azterketan soilik 2.4 M€ gastatu zituen [8].

Nerbioi-Ibaizabal ibaiaren itsasadarra Euskal Herriko itsasadarren paradigma bezala har daiteke. Euskal Herriko gune industrial eta komertzial garrantzitsuena izan da urte askotan zehar (XIX. mendearen bukaeran bertoko meategietan gertatutako burdinaren ustiapenaren ondorioz batez ere). Horren ondorioz aldaketa hidromorfologiko ugari, dragatzeak, portuen eraikuntza eta kutsatzaile desberdinen jaurtitzeak jasan ditu [83, 84]. 1980ko hamarkadan ezagutu zuen itsasadarrak bere egoera larriena. Ondorioz, laster hasi ziren ur haien garbiketarako planak (1984-2009). Momentu horretatik aurrera bere egoera nabarmenki hobetu zen, batez ere bere uretan disolbaturiko oxigenoari eta bertara isurituriko kutsatzaileen kantitateari dagokionez [85, 86].

Uraren Euskal Agentziak argitaratutako datuen arabera, egun *Nerbioi-Ibaizabal* ibaiaren itsasadarreko ur-emari nagusian dagoen metalen kantitatea eta haren ibaiadarretako uretan dagoena parekoak dira. Esan daiteke, beraz, itsasadarraren Garbiketarako Egitasmo Orokorra bere helburua betetzen ari dela [86].

Lan ugari egin dira *Nerbioi-Ibazabal* ibaiaren itsasadarraren egoera ekologikoaren azterketari dagokionez: ura, biota eta sedimentuzko laginak bildu dira itsasadarrean zehar kokaturiko zonalde desberdinetan. Kutsatzaile organikoak zein ez-organikoak neurtu dira laginetan, zenbait propietate fisiko-kimikoekin batera [25, 30, 34, 77, 82, 87-95]. Horretaz aparte uraren egoera biologikoa zehazteko ere zenbait lan burutu dira arrain eta bentos komunitateetan oinarrituz [60].

Lan hauetan guztietan oinarrituz zenbait ondorio atera dira *Nerbioi-Ibazabal* ibaiaren itsasadarraren egungo egoerari buruz: garbiketarako plana hasi aurretik bilduriko datuetan oinarrituz, bere egoera txarra eta eskasaren artean sailkatzen zen. Garbiketarako plana abian jarri ostean haren egoera fisiko-kimikoa nabarmenki hobetu zen, eta prozesu hau nabarmenki azkartu zen Garbiketarako Egitasmo Orokorrak hondakin-uren tratamendu biologikoa martxan jarri zuenean. Horren ondorioz arrainen eta bento komunitateak itsasadarraren goiko partean ere hazten hasi ziren. Hala ere, itsasadarrak konposatu organiko zein ez-organikoen presentzia nabaria du oraindik, bai uretan eta bai sedimentu eta biotan ere. Horregatik nabarmenki eraldaturiko gainazaleko ur moduan (*Heavily Modified Water Bodies*, HMWB) dago sailkatuta une honetan. Beraz, WFD-k eskatzen duen egoera ekologiko ona eskuratzeko tratamendu berezi eta espezifikoaren beharra argia da. Hala ere, kasu berezi honetan nabarmenki eraldaturiko ur-masa baten aurrean gaudela kontuan hartuz, egoera ekologikoaz hitz egin beharrean itsasadarraren potentzial ekologikoa aztertu beharko genuke, hau baita HMWB-etarako benetan definitu behar dena. *Nerbioi-Ibaizabal* ibaiaren itsasadarrean WFD-aren ezarpenerako, beraz, potentzial ekologiko optimoa 2015ean lortzea izango litzateke helburua [96].

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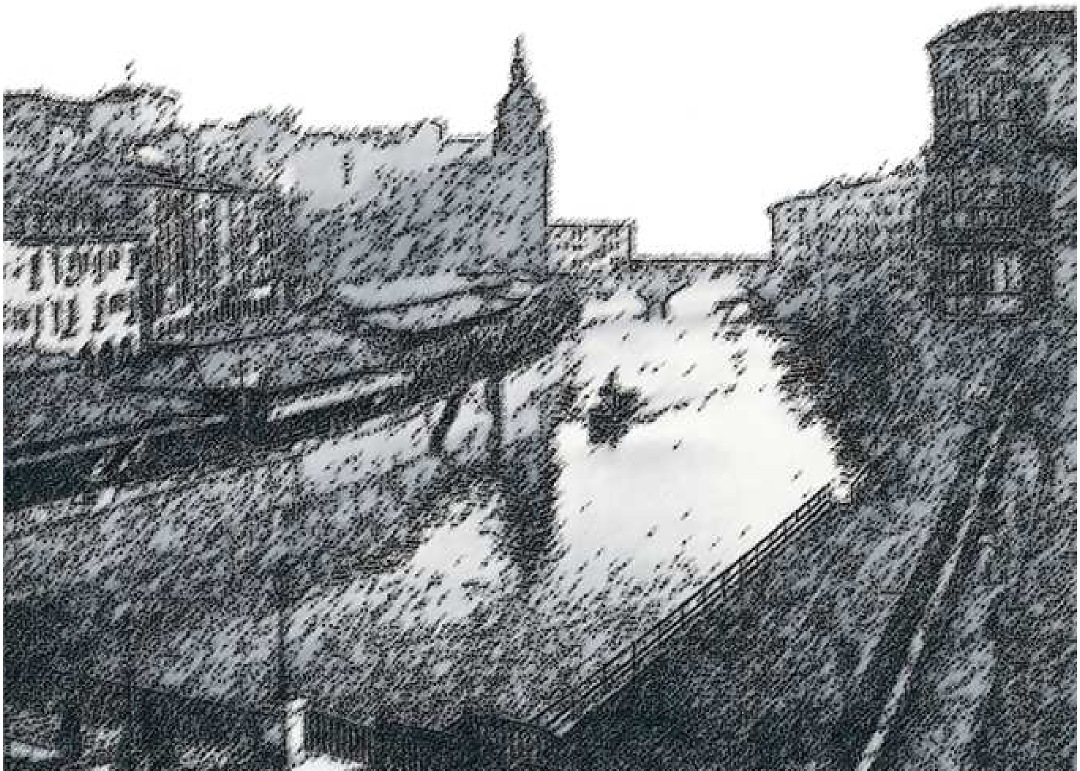
1. Atala

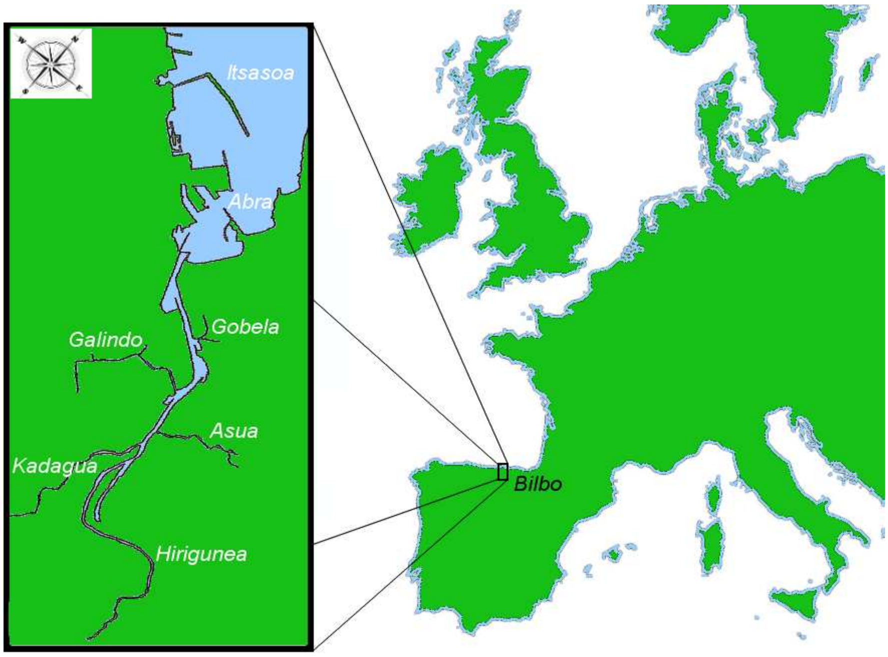
Kutsadura metalikoaren monitorizazioa



Nerbioi-Ibaizabal ibaiaren Itsasadarra: aipamen historikoa eta kutsadura metalikoaren eboluzioa

1. Deskribapen fisikoa

Nerbioi-Ibaizabal Itsasadarra Bizkaiko Golkoaren hego-ekialdean kokatuta dago (43º 20´N, 3º 1´W) (ikusi 1. Irudia). Ibaizabal ibaia eta Nerbioi ibaia Basaurin batzen dira eta elkarketa honetatik eraturiko uharka itsasadar bihurtzen da eta beste lau erreken urak hartzen ditu: Kadagua (%27) eta Galindo (%4) ezkerraldetik, eta Asua (%0.7) eta Gobela (%0.3) eskuinaldetik.



1. Irudia. Nerbioi-Ibaizabal itsasadarren kokapen geografikoa.

Urteko batez besteko gazitasun erlatiboari dagokionez, Europar Batasunak emandako sailkapenaren arabera [1], itsasadarra oligohalino motakoa da (‰0.5-etik ‰5-era) itsasbeheran eta euhalinoa (‰30-etik ‰40-ra) itsasgoran. Mareen batez besteko anplitudea kontuan hartuta, berriz, mesomareala da (2 m-tik 4 m-ra).

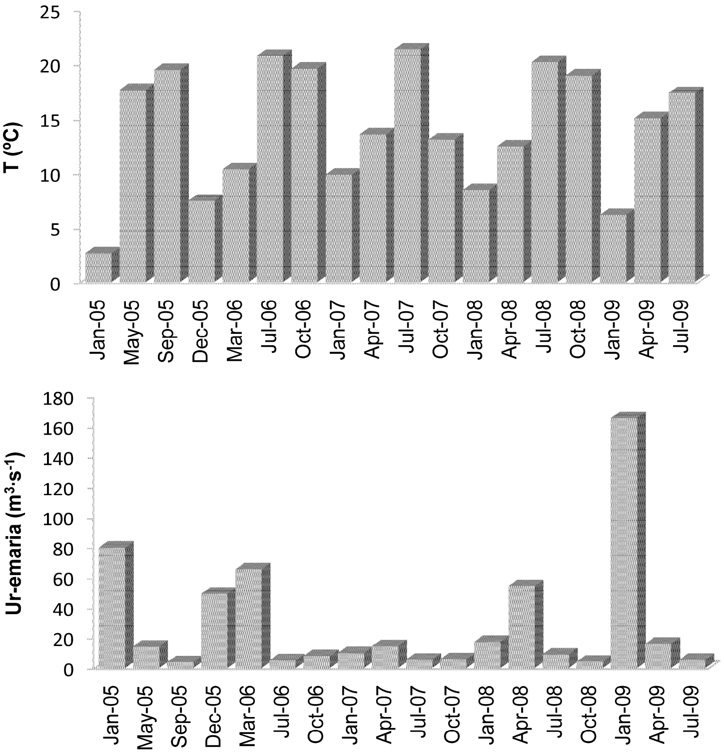
Itsasadarrak 3.5 km-ko zabalera eta 30 m-ko sakonera duen Abra izeneko badian husten ditu bere urak. Itsasadarraren ezkerreko aldean burdinan oso aberatsak diren mendiak daude. Honetaz gain, itsasadarraren azken 15 km-ak nabigagarriak izateak indar handia eman izan dio Bilbo hiriari bere historian zehar.

Itsasadarraren ibai-arroak 1700 km^2-ko azalera du, bere batez besteko fluxua 30 m^3·s^{-1}-koa da [2] eta bere hondoan urtero pilatu egiten den sedimentuen kantitatea 14.000 eta 1.000.000 tona artean dago [3].

Milioi bat biztanleren eragin zuzena jasotzen du, hau da, Bizkaiko biztanleriaren %87a eta Euskal Herriaren %29a. Honetaz aparte, ekaitzak dauden garaietan ur-pilaketak arintzeko ezarritako gainezkabideek (gaur egun 118 inguru dira) presio garrantzitsua eragiten dute itsasadarrean. Azpimarratzekoa da ere Gobela ibaiko ura Abrako badiara eramaten duena euri-erauntsien garaietan. Gainera, itsasadarraren fluxua erregulatzeko eraturiko egiturek (kaiek, eustarriek, eta abarrek) eta portuek era esanguratsuan eraldatu dute bere ibai-ahoa. Nabigazioa eta merkantzien trafikoa errazteko hainbat kai eraiki ziren: Abra badiatik gora abiatuta, Benedikta (ezkerraldean), Lamiako (eskuinaldean), Udondo (eskuinaldean), Axpe (eskuinaldean) eta Portu (ezkerraldean) aurki daitezke. Baina helburu horrekin egindako lanik garrantzitsuena Deustuko kanala da. Kanal itxi honen luzera ezkerreko ertzari dagokionez 3 Km ingurukoa da eta eskumako ertzak, ordea, 2.5 Km ditu. 100 m-ko zabalera du eta itsasadarraren eskuinaldean dago kokatuta, Kadagua ibaiaren ahotik gora 500 m-ra gutxigorabehera.

Kontutan hartu beharra dago, beraz, itsasadarrak, bere osotasunean, super-portu baten modura funtzionatzen duela eta honekin erlazionaturiko presio guztiak pairatzen dituela, besteak beste, lokailuak, ontziolak, kanalerapenak eta dragatzeak. Beste aldetik, hiru kirol-portu txiki daude Abra badian (Santurtzin, Areetan eta Algortan) eta hondakin-urak tratatzeko araztegi handi bat Galindo errekaren ezkerraldean, bere ahotik 500 m-ra eskas.

Nerbioi-Ibazabal itsasadarraren inguruko klima ozeaniko hezea da, tenperatura moderatuekin eta prezipitazio aldetik oso euritsua. Urtaro lehorrik gabeko klima epel honi klima atlantikoa ere deitzen zaio, ozeano Atlantikoak eragin nabarmena duelako. Mendebaldeko haizeen ugaritasunak eta ozeanoaren eragin erregulatzaileak baretu egiten dituzte bere tenperaturak, eta urte osoan zehar klima epela egotea ahalbidetzen dute. Udako batez besteko tenperatura 20 ℃-koa da eta negukoa 8 ℃-koa. Dena dela, urtean hainbat alditan muturreko tenperaturak izaten dira, 30-35 gradutik gorakoak eta 0 ℃-tik beherakoak. Prezipitazioak homogeneoki banatzen dira urtean zehar, baina, normalean ugariagoak izaten dira neguan eta udaberrian [4]. 2. Irudian 2005eko urtarriletik 2009ko uztaila bitartean itsasadarrean buruturiko laginketa-kanpainetako datu hidrometeorologikoak ikus daitezke [5].



2. Irudia. Nerbioi-Ibaziabal itsasadarrean neurtutako tenperatura atmosferikoa (ºC), eta ur-emaria (m^3·L^{-1}) 2005eko urtarriletik 2009ko uztaila bitartean. Tenperatura Nerbio (Abusun), Kadagua (Sodupen) eta Ibaizabal (Amorebietan) ibaien estazio meteorologikoen batez besteko moduan dago kalkulatuta eta ur-emaria hiru ibaien ur-emarien batura da.

2. Zonaldearen historioa

XIV. mendean, Bilbok hiri-titulua eskuratu zueneko garaian, itsasadarraren bi aldeetan nekazaritza eta abeltzaintza ziren bizibide arruntenak. Hala ere, Bilboko hirigunea jadanik merkataritza- eta marinel-gune garrantzitsua zen. XV. eta XVI. mendeetan zehar Bilbo komertzialki indartu zen. Hazkunde ekonomikoa XVI. mendearen amaieran moteldu zen arren, hirigintzak aurrera egin zuen eta horrela jarraitu zuen hurrengo mendeetan zehar.

XIX. mendean hiriaren benetako aldaketa hasi zen. Itsasadarraren ezkerraldeko mendiak burdinan aberatsak zirela aprobetxatuz, Bilbok garapen izugarria izan zuen hurbil zeuden meategien ustiapenaren ondorioz. Honek nabarmenki lagundu zuen itsas-merkataritza eta portuko jarduera sustatzen, siderurgia indarrarekin sortzen zen bitartean. Aurrerapen guzti hauek itsasadarraren inguruko lurraldeen garapen ekonomikoa ekarri zuten eta Bilbo inguruko biztanleria ere izugarri handitu zen [6].

XIX. mendearen erdialdean aduanak lekuz aldatu ziren kostaldera (1841an) eta arantzel berri bat ezarri zen. Hau guztiak, bertoko lurren burdinaren kantitateak eta kalitateak lagunduta, labe garaien irekiera, Boluetan (*Santa Ana*) eta Barakaldon (*Nuestra Señora del Carmen*), bultzatu zuen.

Dinamismo guzti honek bestelako aurrerapenak ekarri zituen hirira, besteak beste, Bilbo beste hiri garrantzitsuekin (Madrilekin, esaterako) elkartzeko bigarren mailako trenbideen eraketa edota Bilboko bankuaren irekiera (1855ean) [7].

Deskribatutako egoera bikainak burdingintzaren garapenerako lehen saiakera bultzatu zuen, 1860. urtean bertan behera gelditu zena. Arrazoi teknikoek, ikatz-mineralaren urritasunak, kapitalisten konfiantza-faltak eta Espainia mailako burdinaren kontsumo txikiak Asturiasen atzean utzi zuten Bizkaia burdin goxoaren ekoizpenean.

Egoera hau, alabaina, 1870. urtean aldatu zen Somorrostro aldeko minerala (fosforo kantitate txikia zuena) Bessemer altzairuaren ekoizpenerako beharrezko bilakatu zenean. Altzairuaren ekoizpenerako sistema berria Europan zehar barreiatu zenean, burdinaren hornitzaile garrantzitsu bihurtu zen Bizkaia. Gainera, 1868. urtean arantzel eta uhasari berrien ezarpenek Estatu Espainiarreko politika ekonomikoa erabat eraldatu zuten. Aldeko egoerak eta Ingalaterrara minerala garraiatzen zuten itsasontzien itzulerako bidaiek kalitate handiko ikatzaren erosketa posible egin zuten eta honekin batera, kalitate handiko burdinaren ekoizpena.

Garai berri horietan, Bilbo inguruko meagintzan eta burdingintzan egindako inbertsioak erabat indartu ziren eta, ondorioz, nazioarteko konpainiak finkatu ziren eskualdean, zuzenean minerala atera eta esportatzeko.

1876. urtetik aurrera (3. karlistada bukatu ondoren) industrian oinarritzen ziren lekuko kapitalak indartu egin ziren eta, ondorioz, zonaldeko inbertsio pribatuak ugaritu ziren. Honek industrian eta meagintzan oinarritutako bertoko burgesia berria ekarri zuen [8]. Aldi berean, Bilbo inguruko herrietan Estatu Espainiarreko biztanle ezberdinez osatutako langileria gero eta ugariagoa zen. Esan daiteke, laburbilduz, garai horietan meagintzan, burdingintzan eta ontzioletan oinarritutako industria erabat finkatuta zegoela Bizkaian, eta kimika-alorrekoa zein metalgintza ere hedatzen hasiak zirela. Garapen-zurrunbilo honetan (XIX. mendearen bukaeran), Bilboko eta honen inguruko herrien garapena bultzatuko zuen komunikazio-bide bilakatu zen Nerbioi-Ibaizabal itsasadarra.

2.1. Bilboko portua

Burdingintzaren garapen azkarra zela eta, 1877. urtean “Junta de Obras del Puerto” izeneko erakundea eratu zen. Bere zereginen artean kaien eta ontzitegien eraketa, eta itsasadarraren uhaska ubidetzea eta dragatzea zeuden. Urteak joan ahala, kai berriak eratu ziren eta, uraren bidezko garraioa hobetu nahian, itsasadarraren sarreran biltzen zen harezko hesia desagerrarazi zen, “Burdin kaia” eratuz, Churruca ingeniariaren eskutik. Honek Santurtziko olatu-horma eraikitzeko obra ere zuzendu zuen.

XX. mendearen hasieran, itsasadarraren barnealdeak 180 hektarea nabigagarri biltzen zituen eta bertan bi zonalde ezberdintzen ziren: portu komertziala, San Antoneko zubitik (Bilboko hirigunetik) Elorrietara joaten zena, eta portu industriala, Portugaleteraino heltzen zena. Azken honek Barakaldo, Sestao eta Erandioko herrietan bilduriko burdingintzak, kimika industrialak eta ontziolak hartzen zituen barne. 1928. urtean karga eta deskargarako kai gehiagoren beharra zela eta, Deustuko Kanala egiteko lanak hasi ziren, baina azpiegitura ez zen 1969. urtera arte bukatu.

80. hamarkadan enpresa ugari itxi zirenez, Bilbo hiriguneko portu komertziala ere itxi egin zen, eta honen ondorioz itsasadarraren portu-instalazio guztiak kanpoaldean, Abran hain zuzen ere, finkatzea erabaki zen. 1991an hasi ziren Abra kanpoaldeko portua handitzeko lanak eta hau Zierbenako portuaren eta Santurtziko olatu-hormaren artean finkatu zen. Era honetan, gaur egun, itsasadarraren barne-trafikoa nabarmenki murriztu da. 70. hamarkadaren bukaeran Bilboko portuan 12 draga, 8 garabi flotatzaile, 29 zirgatzaile, 28 pontoi eta ehun bat gabarra zeuden bitartean, gaur egun draga bakarra, pare bat induskailu, eta dozena erdi bat gabarra besterik ez daude [9].

2.2. Meagintza

XIX. mendearen bukaeran eta XX. mendearen hasieran, aurretik aipatu den bezala, burdinaren esportazioak atzerriko inbertitzaileen jarduera ikaragarri bultzatu zuen. Atzerriko elkarte garrantzitsuenak *Orconera* eta *Franco-Belga* izan ziren eta, ondorioz, meategien ustiapenak ekarritako irabazi gehienak Euskal Herritik kanpo geratu ziren. Enpresa hauek azpiegituretan inbertitu zuten batez ere. Eratu zituzten garraiorako eta kargarako sistemen artean, “Orconera Iron Ore” karga-lekuak oraindik ere zutik dirau, baina Bilboko meagintzaren urrezko urteetan era horretako 20 karga-lekuk ere jarduten zuten.

2.3. Siderometalurgia

Industriaren garapenerako meagintza garrantzitsua bazen ere, benetako arrakasta siderometalurgiari esker heldu zen. XIX. mendearen 80. hamarkadan jatorri Espainiarreko zenbait kapitalistek teknikaren ikuspuntutik puntakoak ziren enpresak ireki zituzten Bilbo inguruan, hala nola, *San Francisco, La Vizcaya* edota *Bilboko Labe Garaiak* (*Altos Hornos de Bilbao).*

Hurrengo urteetan altzairu eta burdinaren ekoizpenerako sistemak berritu ez zirenez, XIX. mendearen bukaerarako Bizkaiko industria teknikoki atzeratuta geratu zen. Faktore honek, beste batzuekin batera, erabat kaltetu zuten Bilboko industria-jarduera, eta Suediaren atzean geratu zen altzairu eta burdinaren ekoizpenaren alorrean.

1922. urtean, Primo de Riveraren diktadura garaian eman ziren aldaketa ekonomiko eta politikoen ondorioz, Bilboko jarduera ekonomikoa erabat berpiztu zen. Baina “urrezko urteak” moduan ezagutzen zen garai oparo hau 1929. urtera arte soilik iraun zuen, nazioarteko krisi ekonomiko handiaren ondorioz eskaria eta produkzioa nabarmenki murriztu baitziren.

Hurrengo urteetan egoera okertuz joan zen pixkanaka, eta 1996. urtean itsasadarrak ezagutu duen siderometalurgiako enpresa garrantzitsuena (*Bizkaiko Labe Garaiak*) publiko bihurtu zen. Guztizko desagerpena saihesteko ahaleginetan, birmoldatu zen gero, *Bizkaiko Altzairutegi Trinkoa* (*Aceria Compacta de Bizkaia)* eratzeko [10].

2.4. Ontzigintza

1887. urtean, gudarako itsasontzien eskaera zela eta, itsasadar inguruko ontziolen jarduera esanguratsuki handitu zen. Altzairuko ontzi-kroskoak eratzen zituen *Astilleros del Nervión* 1890. urtean zabaldu zen. Urte batzuk geroago, 1900an, *Euskalduna de Construcción y Reparación de Buques* konpainia eratu zen.

XX. mendearen erdialdera Europako hirien hazkunde ekonomikoak Bizkaiko ontzigintza indartu eta aberastu zuen, baina 1973. urteko petrolioaren krisiaren ondorioz, sektorea oso kaltetua izan zen, eta pixkanaka ontziolak desagertuz joan ziren.

2.5. Industria kimikoa

Itsasadarraren inguruan industria kimikoa ere asko garatu zen garai batean eta honi esker amonio sulfatozko lehergaien eta ongarrien ekoizle garrantzitsuena bihurtu zen Bizkaia.

Zonaldeko geologiak eskaintzen zuen burdinak, ikatz merkea eskuratzeko erraztasunak eta burdingintzaren bestelako azpiproduktuen eskuragarritasunak, ongarri mineralen (superfosfatoen) ekoizpenean espezializatutako zenbait enpresen irekiera bultzatu zuten, esate baterako, *La Sociedad Anónima Española de la Polvora Dinamita* edo *La Fábrica de Elorrieta.*

XX. mendearen 40. eta 50. hamarkaden artean *Unquinesa* eta *Sefanitro* bezalako enpresa kimikoak ireki ziren [11, 12] baina dibisen eskasia eta atzerriko enpresen konpetentzia zela eta, industria kimikoa ere gainbeheran sartu zen.

2.6. Giza-ihardueraren ondorioak

XIX. mendearen bukaeran hasi zen garapenaren ondorioz, Nerbioi-Ibaizabal itsasadarraren inguru naturala guztiz hondatu zen, bere ertzetan neurririk gabe hedatu baiziren portu, etxe eta industria-enpresa ugari. 3. eta 4. Irudietan honen adibide argia ikus daiteke.

3. Irudia. Bilboko hirigunea industrializazio-garaian.

Bilbo hiriaren populazioak gora egiten zuen neurrian, itsasadarrera zuzenean isuritako hondakin-uren kopurua ere biderkatuz joan zen. Ondorioz Bilboko garapena ekarri zuen komunikazio-bidea bera (itsasadarra, hain zuzen) inguru guztiz kutsatua bilakatu zen. Garai horretan itsasadarrera 900 tona hondakin solido, 400 tona hondakin azido, 80 tona metal astun, tona bat zianurodun konposatu, 600 tona oxigeno eskaera kimiko eta 20 tona nitrogenodun konposatu isuri egiten ziren egunero.

4. Irudia. Deustuko Kanalaren inguruko zonaldea Bilboko industrializazio-garaian.

Egoeraren larritasuna ikusita, hondakin-uren tratamendurako programak garatzea eta ezartzea ezinbestekoa bihurtu zen. Baina industrializazioak ez zuen bakarrik uraren kalitatea okertu, 1960ean biztanlerian eman zen hazkundearen ondorioz, industria eta biztanleria urez hornitzeko arazoak ere izan ziren, batez ere uda partean. Egoera larri honi buelta emateko asmoz, Bilbao-Bizkaiko Uren Partzuergoa martxan jarri zen (1967) [13].

3. Itsasadarraren Saneamendurako Egitasmo Orokorra

1900. urtean saneamendu-sistema aitzindari bat jarri zen martxan, itsasadarraren bi aldeetatik igarotzen ziren bi hodiz osatuta zegoena. Hauek Elorrietan elkartzen ziren eta bilduriko hondakin-urak itsasoan, Punta Galeatik 15km-ra, isurtzen ziren zuzenean. Deustuko kanala eraiki zenean aipatutako saneamendurako sistema erabat baztertuta geratu zen eta hondakin-urak itsasadarrera isurtzen hasi ziren zuzenean. Hori dela eta, Bilboko itsasadarra Europako kutsatuenetarikoen artean kokatu zen.

1979ko “Bilboko Itsasadarraren Saneamendurako Egitasmo Orokorra”-k ondoko helburuak ezarri zituen: ur-hornikuntza ziurtatzea, itsasadarraren zonalde guztietan bizitza berreskuratzea, hondartzak berreskuratzea eta isuri-industrialen kantitatea nabarmenki murriztea. Itsasadarraren edozein puntutan eta edozein unetan disolbatutako oxigenoaren portzentajea, gutxienez, %60koa izatea ezarri zen helburu orokor bezala [13].

Azkenean, eta aukera ezberdinen artean, udalerrietatik zetozen estolderiak elkartuko zituzten zortzi hodi eraikitzea erabaki zen eta, hauekin batera, lokatz aktibatuen bidezko tratamendu biologikoa zuten lau araztegi, Boluetan, Galindon (5. Irudia), Lamiakon (gaur egun eraikitze-lanetan) eta Muskizen. Galindoko araztegia garrantzitsuena da eta Bizkaiko biztanleriaren %80ak eraturiko hondakin-urak jasotzen ditu, urtean 125 milioi metro kubiko inguru.

5. Irudia. Galindoko araztegia.

Baina araztegira ez da ura soilik heltzen, honekin batera hondakin-organikoak, egur-zatiak, lokatza (85.000 tona) eta bestelako objektuak heltzen dira. Lokatza sikatu eta, beharrezko tratamendu biologikoa burutu ostean, beste hondakin-solidoekin batera erretzen da. Eratzen diren 10.000 tona errauts lehengai moduan erabiltzen dira porlan-fabriketan [14].

Lan guzti hauek, industria kutsakorrenen eta meategien itxierarekin batera, fruituak eman dituzte. Gaur egun, ezarritako helburuak bete direla esan daiteke. Uretan lortu den oxigenoaren mailarekin itsasadarrean gora egiten du etengabe aktibitate biologikoak [15]. Aipagarria da, adibidez, muturreko aktibitate-industriala garatu zen zonaldeetan (Zorrotzan, Deustun eta Axpen esaterako) 1990 eta 2002. urte bitartean uretan disolbaturiko oxigeno portzentajea %25 ingurukoa izatetik %60-%70 tartekoa izatera pasa zela. Uren kalitatean jazotako aldaketaren adibide gisa 6. Irudia dugu.

6. Irudia. Bizkaiko Zubia industrializazio-garaian eta gaur egun.

Gainera, itsasadarreko gune asko berreskuratu dira aktibitate ludikoetarako (arrantzarako eta kirolarako, besteak beste) eta Abra inguruko hondartzen kalitatea nabarmenki hobetu da. 7. Irudia Bilbon 2011ko Maiatzaren 15ean ospatu zen igeriketa-txapelketari dagokio.

Urte askotako kontrol gutxiko ustiapen industrialaren aztarna, alabaina, itsasadarrean geratu da. Beste edozein itsasadarretan gertatzen den bezala, Nerbioi-Ibaizabal itsasadarreko sedimentuak garai horretako lekuko dira.

Izan ere, kutsatzaileen azken biltegia itsasadarretan beheko eta erdiko zonaldeko sedimentua da, geruzaz geruza, urtetan zehar isuritako kutsatzaileak bertan pilatzen baitira. Kasurik hoberenean, kutsatzaileak sedimentuan finkatzen dira, espezie kimiko egonkorrak eratuz. Baina, itsasadarreko unean-uneko ezaugarri fisiko-kimikoen arabera edo bat-bateko gertakizun naturalen (euri-erauntsien) edo ez-naturalen (industria-isurkinen, dragatze-lanen, eta abarren) ondorioz, pilatutako kutsatzaileak bermobiliza daitezke bioeskuragarri bihurtuz eta giza-osasunerako ere arazo larri bilakatuz [16]. Hortaz, itsasadarretako sedimentuek duten kutsatzaileen (eta konkretuki metalen) kontzentrazioaren jarraipenak duen garrantzia.

7. Irudia. Bilbon ospatutako “Training Day”-a (El mundo, 2011ko Maiatzaren 15ean).

4. Itsasadarraren inguruan buruturiko aurretiko ikerketa-lanak

Nerbioi-Ibaizabal ibaiaren itsasadarraren historia kontutan hartuta, asko dira inguru honetan kutsaduraren izaera, banaketa geografikoa eta denborarekiko bilakaera aztertzeko burutu diren ikerketak. Lan honetan kutsadura metalikoa aztertu denez, sedimentu eta uretan espezifikoki, horretan dago zentratuta hurrengo pasarteetan egin den eztabaida.

Sedimentuen eduki metalikoari buruz egindako lanak ugariak direla ikus daiteke; urari buruz argitaratutakoak, berriz, askoz urriagoak. Lehenengoei dagokienez, era askotako lanak burutu dira. Aitzindaria Seebold-ek, Labartak eta Amigók 1982an argitaratu zutena da [17]. Artikulu horretan titanioaren eta beste metalen distribuzioa ikertzen da itsasadarrean. Beranduago, Eusko Jaurlaritzak 1998ko urtarrilean itsasadarraren 25 puntutan bildutako sedimentuetan aurkitutako hainbat metalen kontzentrazioak argitaratu zituen [18].

Zaintzarako laginketa-saio luzeetan oinarrituz, itsasadarreko sedimentuetako metalen kontzentrazioan azken urteetan gertatutako espazioarekiko eta denborarekiko bilakaera aztertu da [19-22]. Sedimentuetan pilaturiko elementu metalikoen eragin biologikoen azterketa ere burutu da, SQG bezalako gidak erabiliz [19, 20, 23]. Bestetik, traza-mailako elementuen erauzketa-prozesuak fokatutako ultrasoinuzko eta mikrouhinezko energiaren bidez azkartzea eta optimizatzea lortu da itsasadarreko sedimentuak erabiliz [24, 25]. Merkurio eta bere espezieen (metilmerkurioaren, bereziki) ugaritasunaren azterketa zehatzak burutu dira. Merkuriozko espezieen presentzia sedimentuetan beste itsasadar batzuetan lortutakoekin konparagarriak diren arren, Nerbioi-Ibaizabalerako estimatutako balio naturaletatik gora daude nabarmenki [26-29]. Artsenikoaren espeziazioa eta portaera itsasadarraren konpartimendu ezberdinetan (uretan, sedimentuan edo esekidurako partikuletan) zehazteko saiakerak egin dira erauzketa-prozesu ezberdinak erabiliz [30]. Itsasadarraren kutsaduraren eboluzio historikoa ikertzeko, sakontasun handiko sedimentuzko zutabeak datatu eta analizatu dira, foraminifero bentikoen presentzia eta metalen kontzentrazioak aztertuz [22, 31, 32]. Lan hauetariko batean hainbat elementuren balio naturalak estimatu ziren zonalderako [31]. Beste bide batzuk ere erabili dira balio naturalak estimatzeko, oro har, maila bereko emaitzak lortuz [33]. Adierazle biologikoak [34, 35] eta biomarkatzaileak [36] ere erabili dira itsasadarraren kutsadura metalikoa aztertzeko. Metalen iturri posibleak itsasadarrean zehar determinatzeko lanak ere aipagarriak dira [2]. Azkenik, zaintzarako monitorizazio-saio luzeak diseinatzeko epe laburreko laginketak ere garatu dira, zonaldearen aurretiko ezagutza ahalbideratu dutenak [2].

Urari dagokinez, WFD-ren aginduak jarraituz eta estatistikan oinarrituz, zenbait saiakera egin dira itsasadarreko metalen balio naturalak uretan estimatzeko [37]. Saneamendurako Egitasmo Orokorrak uren kalitatean izandako eragina ere aztertu da, emaitza positiboak lortuz [13, 15, 21]. Bestetik, epe luzeko monitorizazio-saiotan kutsadura metalikoaren denborarekiko eta espazioarekiko eboluzioa ikertu da itsasadarraren uretan [38]. Metalen presentzia uretan munduko beste itsasadar batzuekin konparagarria izan arren, eta behaturiko denboraren araberako eboluzioa onerako izan arren, itsasadarra nabarmenki eraldaturiko gainazaleko ur moduan (*Heavily Modified Water Bodies*, HMWB) sailkatu behar da oraindik [39]. Azkenik, itsasadarreko puntu ezberdinetan uretan neurturiko traza-mailako elementuen kontzentrazioak behatuz, kutsadura metalikoaren balizko iturriak zehazteko lanak garatu dira, jatorri antropogenikoko hainbat elementu (As, Cd, Pb, Sn, Zn, Cu eta V) identifikatuz [38, 40].

Hala ere, itsasadarraren sedimentuen zein uren kutsadura metalikoa aztertzeko egindako monitorizazio-saioen diseinua sinple samarra izan da kasu gehienetan. Salbuespenak salbuespen, laginketa-puntuen kopurua mugatua izan da eta/edo laginketen maiztasuna eskasa eta/edo laginketen denborarekiko iraupena laburra. Laginketa-puntuen urritasunaren arrazoiak bi izan dira kasu gehienetan: i) aldibereko lana burutzea ingurune desberdinetan edo ii) laginak geografikoki sakabanatuta dauden puntuetan hartu beharrean, puntu bakar batean biltzea sakonera desberdinetan, kutsadura historikoa ikertzeko nahian. Buruturiko ikerketa-lanetan, gainera, metalen determinaziorako erabilitako analisi-metodoak, kasu gehienetan, ez dira bat etorri. Hau guztiak emaitzen arteko konparaketa erabat oztopatzen du [41]. Hala ere, eta aipatutako artikuluen emaitzak aztertu ondoren, oso modu orokorrean esan daiteke Nerbioi-Ibaizabal ibaiaren itsasadarrean kutsadura metalikoak etengabe egin duela behera 1997tik 2007ra doan hamarkadan [19, 42].

5. Lan honen zenbait zehaztasun

Aipatutako guztia kontutan hartuta eta WFD-aren gomendioak jarraituta, Nerbioi-Ibaizabal itsasadarrean kutsadura metalikoaren inguruan gure ikertaldeak 2008ko urrira arte arte burutu izan duen lana osatu nahi izan dugu, besteak beste, 2005ean hasitako zaintzarako-monitorizazioari segida emanez. Hurrengo hiru ataletan, azken hiru urteetan (2008tik 2010rako tartean) arlo horretan egindako lanaren emaitzak aurkezten dira. Lehen eta bigarren ataletan, hurrenez hurren, 2008tik 2010era bildutako ur-laginen eta sedimentuen analisien emaitzak bildu dira. Kutsaduraren denborarekiko eboluzioa ikertzea ahalbideratu dute emaitza hauek. Hirugarrenean, berriz, 2009ko eta 2010eko urtarrilean egindako makrolaginketen emaitzak laburbildu dira. Itsasadarraren 49 puntutan batu ditugun sedimentuen analisiak kutsadura metalikoaren egungo banaketa geografikoa zehaztasun handiagoz ezagutzeko aukera eman digu. Zaintzarako monitorizazioari buruzko informazio orokorra hurrengo pasarteetan aurki daiteke.

5.1. Laginketa puntuen kokapena

Itsasadarraren kontaminazioa sakonki aztertzeko epe luzeko monitorizazio-saioak aurrera eramatea beharrezkoa dela jakina da. Zeregin honetan arrakasta izateko funtsezkoa da zonaldearen adierazgarriak diren laginketa-puntuak aukeratzea. Lan honetan bai gure ikerketaldeak zein beste talde batzuk zonaldean iraganean buruturiko lanak kontuan hartu dira laginketa-puntu ordezkagarrien sorta aukeratzeko. Hautaketa honetan, puntuetara heltzeko erraztasun-maila ere kontuan hartu da.

Hau guztia kontsideratuz, zortzi puntu aukeratu dira itsasadarrean. Horietako lau ibaiadar nagusienen (Kadaguaren, Asuaren, Galindoren eta Gobelaren) arroan daude kokatuta, beste bat bokalean (Arriluzen) eta beste bi, aldiz, ibaiaren uharka nagusian, Alde Zaharrean eta Zorrotzan. Azkeneko puntua, Udondo, kai erdi-itxi batean dago. 1. Taulan laginketa-puntuen kokapen geografikoa eta kodeak bildu dira.

5.2. Laginketen maiztasuna

Aurretik aipatutako zortzi puntuetan ura (gainazalean eta hondoan, itsasgoran eta itsasbeheran) eta sedimentua bildu ziren 2008ko apirilatik 2010eko urrira doazen 11 laginketa-kanpainetan. Metodologia berdina jarraitu zen aurreko lan batean [42] 2005eko urtarriletik 2008ko urtarrilera doan tartean. Denborazko serie luzea da (bere osotasunean hartu da kontuan lan honetan, 2005etik 2010era), denborarekiko edota urtaroaren araberako balizko aldaketak antzematea ahalbideratuko lukeena.

1.Taula. Laginketa puntuen kokapen geografikoa eta beraien identifikazioan erabilitako kodeak.

LAGINKETA PUNTUA	**KOKAPEN GEOGRAFIKOA**
ARRILUZE (AR)	N 43º 20´15,6´´;W 03º 00´38,4´´
GOBELA (GO)	N 43º 19´04,5´´;W 02º 59´35,1´´
UDONDO (UD)	N 43º 18´55,8´´;W 02º 59´30,0´´
ASUA (AS)	N 43º 17´35,8´´;W 02º 58´04,2´´
GALINDO (GA)	N 43º 18´14,4´´;W 02º 58´58,0´´
KADAGUA (KA)	N 43º 16´40,8´´;W 02º 58´37,6´´
ZORROTZA (ZO)	N 43º 16´04,1´´;W 02º 57´35,9´´
ALDE ZAHARRA (AZ)	N 43º 15´38,4´´;W 02º 55´30,4´´

Laginak hiru hilero bildu ziren, urtaro bakoitzean gutxi gora behera. Laginketa-kanpainen data zehatza eta bakoitzean bildutako lagin-motak 2. Taulan ikus daitezke (kasu gehienetan, laginketa bakoitzaren iraupena bi egunekoa izan da, baina taulan soilik lehenengo eguna dago adierazita).

2.Taula. Laginketa-kanpainen datak eta hauetan bildutako laginak
(I.B.: Itsas-behera; I.G.: Itsas-gora).

kanpaina	Eguna	Gainazaleko ura		Hondoko ura		Sedimentua
		I.B.	*I.G.*	*I.B.*	*I.G.*	
05/01	**05-01-26**			√	√	√
05/05	**05-05-25**			√	√	√
05/09	**05-09-05**			√	√	√
05/12	**05-12-15**			√	√	√
06/03	**06-03-16**	√	√	√	√	√
06/07	**06-07-11**	√	√	√	√	√
06/10	**06-10-09**	√	√	√	√	√
07/01	**07-01-17**	√	√	√	√	√
07/04	**07-04-23**	√	√	√	√	√
07/07	**07-07-12**	√	√	√	√	√
07/10	**07-10-24**	√	√	√	√	√
08/01	**08-01-21**	√	√	√	√	√
08/04	**08-04-17**	√	√	√	√	√
08/07	**08-07-01**	√	√	√	√	√
08/10	**08-10-13**	√	√	√	√	√
09/01	**09-01-26**	√	√	√	√	√
09/04	**09-04-22**	√	√	√	√	√
09/07	**09-07-06**	√	√	√	√	√
09/10	**09-10-21**	√	√	√	√	√
10/01	**10-01-14**	√	√	√	√	√
10/04	**10-04-26**	√	√	√	√	√
10/07	**10-07-15**					√
10/10	**10-10-26**	√	√	√	√	√

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Long-term monitoring trace elements in estuarine waters; A case study: The estuary of the Nerbioi-Ibaizabal River (Bilbao, Basque Country)

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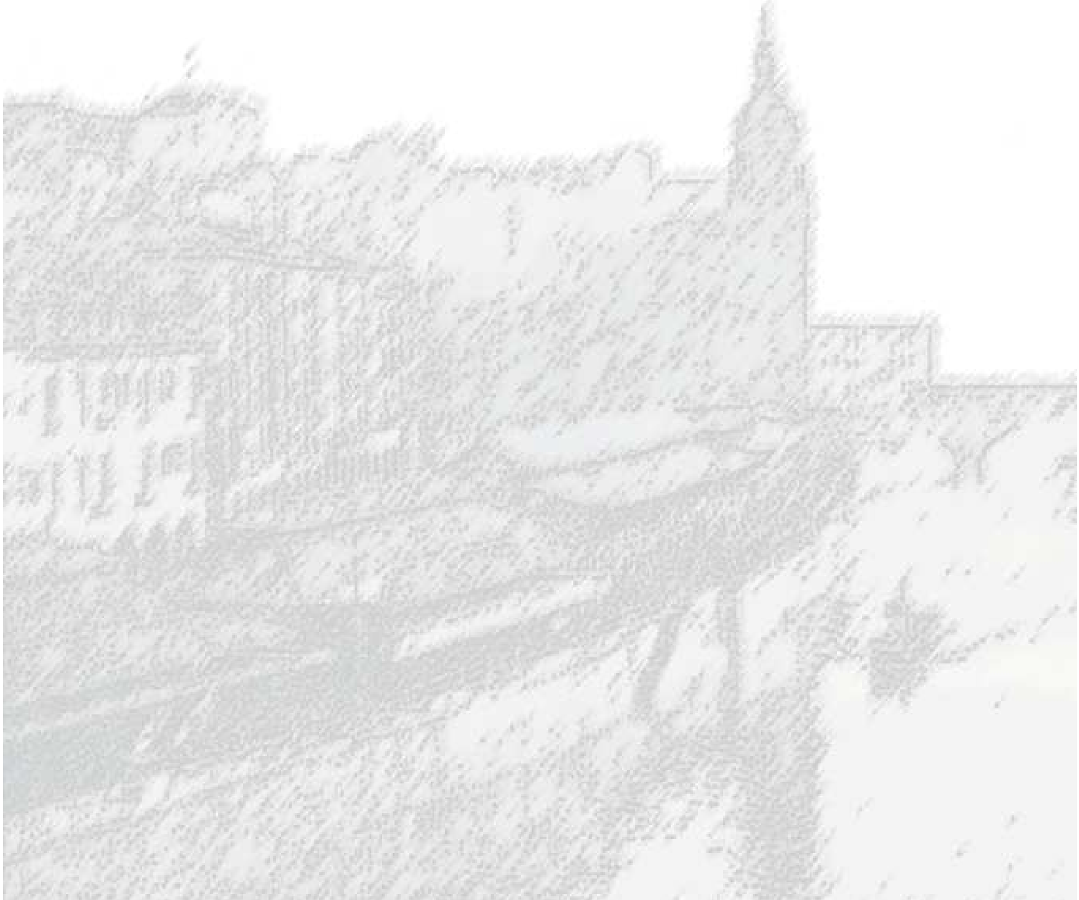

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Abstract

Estuaries are usually highly affected by pollutant discharges of both urban and industrial origin. Environmental risk assessment studies in estuaries most often include trace elements due to their high toxicity and regular presence in this kind of discharges. The fate of trace elements in an estuary is clearly influenced by changes in physico-chemical properties of its waters due to the tidal effect. Rigorous monitoring programs are necessary for the adequate study of the spatial distribution and evolution in time of trace elements in estuarine waters. These programs should include factors such as salinity, sampling depth, proximity to pollution inputs and sampling season. In this work we describe a monitoring program carried out in the estuary of the *Nerbioi-Ibaizabal* River (metropolitan area of Bilbao, Basque Country) to investigate the fate of several trace elements in water. This estuary is located in one of the most populated (~1 million) areas on the Bay of Biscay, with numerous industrial, urban and recreational activities. The exploitation of local iron at the end of the 19th century and ship-building in the bay also have had clear influence on the water quality of the estuary. Surface and deep (in contact with the sediment) water samples were collected approximately every three months from January 2005 to October 2010 (22 sampling campaigns) at eight different points of the estuary (four in tributary rivers, one in an enclosed dock area, two in the main channel, and one in the mouth of the estuary). The sites were sampled at low and high tides. Physico-chemical parameters such as temperature, redox potential, dissolved oxygen and conductivity were measured in situ by means of a multi-parameter probe. The values found were typical from a highly stratified estuary; the average dissolved oxygen (DO) concentration was ~65% of saturation. Concentrations of Al, As, Cr, Cu, Fe, Mn, Ni and Zn, measured simultaneously in the samples by inductively coupled plasma/mass spectrometry (ICP/MS), were comparable to those of similar estuaries in Europe, with the exception of As, Cu and Fe, which presented significantly higher values. Concentrations of most of the metals at most of the sampling sites significantly increased with time over the period investigated, a trend that was especially noticeable in samples collected at high tide at the bottom. Decreasing trends were found systematically in time series of pH, whatever the sampling site and the characteristics of the samples (deep, surface, high tide and low tide). Possible sources of each metal to the estuary were also investigated.

Keywords: Trace elements, estuaries, Nerbioi-Ibaizabal, Bilbao, estuarine waters, monitoring programs, risk assessment.

1. Introduction

Various definitions have been proposed in the literature to describe estuaries adequately. Most of them agree that estuaries are semi-enclosed transitional water systems with a permanent tidal effect, in which sea water is diluted with fresh water from rivers (Fairbridge, 1980; Liu et al., 2003; Caruso et al., 2010).

Because of their complex hydrodynamics (Zwolsman and Van Eck, 1999), coupled with shallow depth and tidal influence, estuaries are highly variable water bodies, with strong spatial and temporal variations (Batlle-Aguilar et al., 2007). These variations affect the physico-chemical properties of estuaries, producing vertical and lateral gradients in salinity, pH, suspended particulate matter, DO and organic and inorganic nutrients (Nolting et al., 1999; Abowei, 2009; Schäfer et al., 2009). Salinity gradients, for example, play a fundamental role in the biogeochemical, physical and biological processes of estuarine systems (Zwolsman et al., 1997; Chaudry and Zwolsman, 2008; Fernández et al., 2008). The pH also has direct influence in chemical reactions associated with the formation and dissolution of minerals, and depends on the presence of organic acids and the extent of biological and physical processes (Directorate, 1979).

With close connections to land and open sea, estuaries are favorable environments for propagation and growth of many organisms (Bierman et al., 20100). Apart from their ecological importance, estuaries throughout history have been notable economic resources, as centres of industrial and urban activities, among other uses. Moreover, estuaries provide convenient routes for transportation and conduits for disposal of wastewaters (Spencer, 2002). For all of these reasons they are considered the most productive coastal environments, even more than the open sea.

Although the evaluation of the ecological status of coastal marine ecosystems has grown significantly since the 1990s, the Water Framework Directive (WFD 2000)—for restoring the quality of surface and ground waters in aquatic systems within the European Union—was not introduced until October 2000. Because information about background levels of dissolved metals in waters is scarce (Tueros et al., 2008; Zaldívar et al., 2008), water quality indexes (WQI), flow rates, tidal currents, and degradation coefficients have been used as surrogates to assess water environmental capacity[2] (Sánchez et al., 2007; Periáñez, 2009; Li et al., 2010). Monitoring programs are, however, the most popular tools to fulfill this task. Regular monitoring programs provide useful, representative and specific information about water quality (Devlin et al., 2007; Sánchez et al., 2007).

A variety of parameters is typically monitored in estuaries. Physico-chemical variables, such as the above-mentioned DO, conductivity and pH, are commonly used as pollution indicators (Micheletti et al., 2001; Raj and Azeez, 2009). Apart from these, other parameters related to anthropogenic inputs are often measured, because these inputs can damage severely the environmental capacity of transitional waters. Concentrations of persistent organic pollutants and inorganic substances (trace elements principally) are commonly quantified (Grant and Middleton, 1990; Williams and Benson, 2009; Grifoll et al., 2010). Specifically, anthropogenic inputs of metals come principally from tributary rivers, direct discharges associated mainly with urban wastes, mining and industrial activities, and atmospheric deposition (Deepti et al., 2009). All of these inputs, as free metal ions in solution, are easily accumulated by aquatic organisms (Vicente-Martorell et al., 2009; Wallner-Kersanach et al., 2009) or adsorbed on suspended matter, and finally removed to the sediments (Spencer, 2002; Ip et al., 2007; Fdez-Ortiz de Vallejuelo et al., 2010b). Although sediments are potentially more hazardous, as the result of their capacity for accumulating trace elements, water analyses provide data for metal concentrations in a specific area at a particular moment.

Following the recommendations of the WFD, in this work we have carried out surveillance monitoring of metal pollution in waters of the estuary of the Nerbioi-Ibazabal River. This estuary is located in one of the most populated areas on the Cantabrian coast (~1 million people) and has suffered throughout its history abundant industrial, mining and urban activities, which significantly degraded its environmental quality (Belzunce et al., 2001; Cearreta et al., 2002; Fernández et al., 2008; Tueros et al., 2008; Fdez-Ortiz de Vallejuelo et al., 2009). The extent of metal pollution in sediments of the estuary has been investigated rather thoroughly (Cearreta et al., 2002; Sanz Landaluze et al.,

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[2] Environmental capacity is defined as “a property of the environment and its ability to accommodate a particular activity or rate of an activity, such as the discharge of contaminants, without unacceptable impact” (GESAMP 1986).

2004; Bartolomé et al., 2006; Raposo et al., 2006; Moros et al., 2009; Fdez-Ortiz de Vallejuelo et al., 2010b; Fdez-Ortiz de Vallejuelo et al., 2010a; Moros et al., 2010), but analogous studies of its waters are scarce (Borja et al., 2004; Borja et al., 2006; Fernández et al., 2008).

An intensive monitoring program carried out from January 2005 to October 2010 is described in this work. Deep and surface water samples were collected—at high and low tides—at eight points of the estuary every three months. Physico-chemical parameters, i.e. temperature, conductivity, pH, redox potential and dissolved oxygen were measured in situ. Concentrations of Al, As, Cr, Cu, Fe, Mn, Ni, and Zn were measured also. The results allowed us to investigate spatial and temporal trends in the dataset, and to draw some conclusions about possible pollution sources in the estuary.

2. Materials and methods

2.1. Study area

The estuary of the Nerbioi-Ibaizabal River is located on the continental shelf of the Cantabrian Sea in the south-eastern corner of the Bay of Biscay. It is 15 km long, and although its principal freshwater input comes from the Nerbioi and Ibaizabal Rivers, it also receives contributions from four tributaries: Kadagua (27%), Galindo (4%), Asua (0.7%), and Gobela (0.3%) (Leorri et al., 2008). The average flow of the estuary is about 30 $m^3 \cdot s^{-1}$, discharging into Abra Bay, which is 30 m deep and 3.5 km wide (Belzunce et al., 2001). According to the annual average salinity, the estuary is classified as oligohaline at low tide and as euhaline at high tide (WFD, 2000).

As a consequence of mining and industrial activities starting in the last decades of the 19th century, the estuary was extremely affected by uncontrolled releases of organic and inorganic pollutants (Saiz-Salinas, 1997; Belzunce et al., 2001; Cearreta et al., 2002). Moreover, to make navigation easier, the original channel was significantly modified. However, the condition of the estuary improved significantly at the end of the 20th century because most of the industries were closed and, starting in 1984, an ambitious plan for the environmental recovery of the estuary was implemented (Barreiro and Aguirre, 2005; Fdez-Ortiz de Vallejuelo et al., 2010b).

2.2. Sampling and analysis

Surface (top 10 cm) and deep (in contact with the sediment) water samples were collected at eight different locations along the estuary (Figure 1), both at low and high tides. Two of the sampling sites were located in the main channel (*ZO* and *AZ*), one in the mouth of the estuary (*AR*), another in a semi-enclosed dock area (*UD*), and the rest were located in the tidal part of the principal tributaries (*GO*, *AS*, *KA* and *GA)*. The sites were sampled every three months from January 2005 to October 2010, a total of 22 sampling campaigns. Deep water at high tide was collected only at one site (*ZO*) in the last seven sampling campaigns. It had been observed in previous studies (Fernández, 2008) that the characteristics of deep water at high tide were very similar all over the estuary.

Figure 1. Estuary of the Nerbioi-Ibaizabal River and location of the eight sampling stations: Arriluze (*AR*), Gobela (*GO*), Udondo (*UD*), Galindo (*GA*), Asua (*AS*), Kadagua (*KA*), Zorrotza (*ZO*) and Alde Zaharra (*AZ*).

Trace element analysis in natural waters requires the use of careful procedures to avoid sample contamination. All the material in contact with the samples was firstly cleaned with Milli-Q water (κ <0.05 $\mu S \cdot cm^{-1}$, Millipore, Billerica, MA, USA), then immersed in a 10% HNO_3 bath for 24 h, and finally soaked again with Milli-Q water. When necessary, the pre-cleaned material was dried in an oven at 50 °C.

Water was collected with a 2 L Van Dorn type all-plastic water sampler (KD Denmark, Research Equipment, Silkeborg, Denmark), specifically designed for trace metal sampling. After homogenizing and filling a 0.5 L Pyrex bottle with the sample, it was protected against light using aluminium foil and transported to the lab in a cool box. Procedural blank samples with Milli-Q water were handled in the same way. Temperature (°C ± 0.1), redox potential (mV ± 0.1), DO (% of saturation ± 0.1) and electrical conductivity ($mS \cdot cm^{-1}$ ± 0.01) were measured in situ using a YSI 556 multi-parameter probe (YSI Environmental, Yellow Springs, OH). Salinity and total dissolved solids (TDS) were estimated from the conductivity measurement.

Once in the laboratory, the water samples were filtered through 0.45 µm cellulose filters (Whatman) in a pre-cleaned standard borosilicate vacuum system and acidified with HNO_3 to an approximate concentration of 1%. The acidified samples were stored in 50 mL polyethylene vials at 4 °C and protected against light until analysis. Concentrations of Al, As, Cr, Cu, Fe, Mn, Ni, and Zn were measured simultaneously by ICP/MS (Elan 9000, Perkin Elmer, Ontario, Canada), using the external calibration method with internal standard correction (Be, Sc, In and Bi) in a Class 100 clean room. The calibrants and sample dilutions were prepared on a mass basis. The operating conditions used in the ICP/MS measurements are summarised in Table 1.

Table 1. Experimental conditions used in the analysis by ICP/MS.

Nebulisation flow	$0.94\ L \cdot min^{-1}$
Plasma flow	$15\ L \cdot min^{-1}$
Auxiliary flow	$1.2\ L \cdot min^{-1}$
Sample flow	$1\ mL \cdot min^{-1}$
Measured isotopes	^{27}Al, ^{75}As, ^{52}Cr, ^{63}Cu, ^{57}Fe, ^{55}Mn, ^{60}Ni, ^{66}Zn
Radiofrequency power	1000W
Integration time	1000 ms
Replicates	3-4
Cones material	Ni
Internal Standards	^{9}Be, ^{45}Sc, ^{115}In, ^{209}Bi

Further details on the experimental procedure can be found elsewhere (Fernández et al. 2008). The accuracy of the method was checked by triplicate analysis of the NIST 1640 certified reference material (natural water, National Institute of Standards and Technology) with satisfactory results (Figure 2). The detection limits (in $\mu g \cdot kg^{-1}$) estimated for the analytes (3 times the standard deviation of 8 replicates of the blank) were as follows: Al, 2.7; As, 0.6; Cr, 0.8; Cu, 1.2, Fe, 18; Mn, 0.1; Ni, 0.6; and Zn, 2.1.

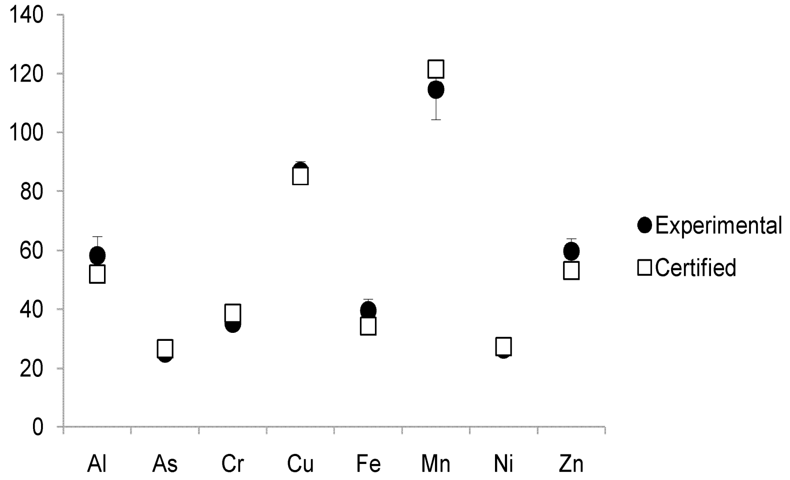

Figure 2. Trace element concentrations found after triplicate analysis (n=3) of the NIST 1640 reference material (black circles), together with the corresponding certified values (white squares).

3. Results and discussion

3.1. Physico-chemical parameters

3.1.1. Spatial distribution

The physico-chemical variables were all measured in deep and surface water at low and high tide. Average, maximum and minimum values detected in each campaign are summarized in Tables 2-5.

As expected, water temperature exhibited seasonal variation (4.0-26.2 °C), but there was no significant difference among sites within each sampling period. The lowest values usually were observed in January at high tide in the bottom water. As a consequence of the huge wastewater treatment plant 100 m upstream from the *GA* sampling point, slightly higher temperature was recorded routinely at this point, especially in winter and in surface water samples.

The pH of an aquatic system is determined by several factors, such as dissolution of carbonate rocks, atmospheric CO_2 exchange, and respiration of aquatic organisms (Mosley et al., 2010). Values of pH from 6.0 to 8.6 are typical for estuarine waters. No significant difference was observed among the pH values measured in samples collected at different points, but the highest were observed regularly in surface waters. The range of pH was 6.1–8.5, within that defined for unpolluted estuarine waters (ANZECC/ARMCANZ, 2000; Spiteri et al., 2008; Abowei, 2009; Williams and Benson, 2009).

Salinity and total dissolved solids (TDS) were calculated from conductivity measurements, and the trends observed for conductivity were extrapolated to the other two variables. As expected, samples collected at high tide always exhibited significantly higher conductivity than those collected at low tide. Furthermore, because denser marine water flows in the deeper portions of the channel, deep water collected at high tide showed the highest conductivity, with maximum values close to 50 mS·cm^{-1}. No significant differences were observed among the conductivities measured in deep waters at high tide along the estuary. Conversely, important spatial variability was clearly distinguished in the conductivity of surface samples, especially at high tide (Figure 3a).

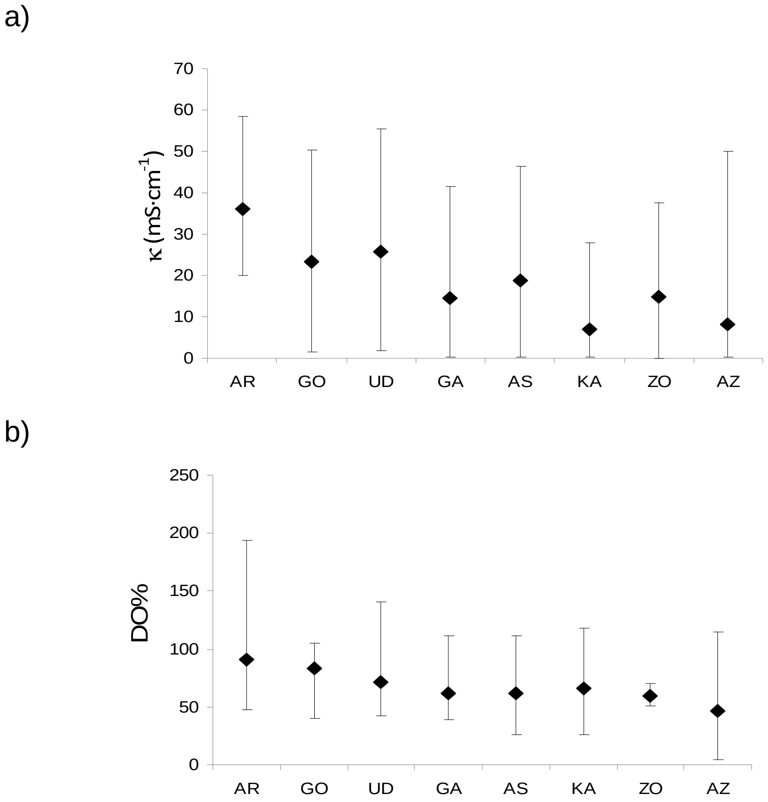

Figure 3. a) Average (black point), maximum and minimum conductivity values (mS·cm^{-1}) measured in surface water samples collected at high tide at each sampling site from May 2005 to October 2010; b) Average (black point), maximum and minimum dissolved oxygen concentrations (DO, expressed as % of saturation) measured in deep waters collected at low at each sampling site from January 2005 to October 2010

Table 2. Average, maximum and minimum temperature (T), pH, electrical conductivity (κ), dissolved oxygen (DO), redox potential (ORP), total dissolved solids (TDS) and salinity (SAL) found in each sampling campaign at high tide in deep water. NA is not analysed.

Campaign	**T(ºC)**	**TDS(g·L^{-1})**	**SAL(g·L^{-1})**	**DO (% of saturation)**	**κ (mS·cm^{-1})**	**pH**	**ORP(mV)**
Jan-05	NA	NA	NA	NA	NA	NA	NA
May-05	16.2 (17.2 - 15.1)	24.13 (27.27 - 15.67)	23.6 (27.0 - 14.7)	56.1 (75.5 - 22.6)	37.12 (41.94 - 24.11)	7.84 (7.97 - 7.54)	161.5 (231.0 - 101.3)
Sep-05	22.0 (24.6 - 20.8)	31.83 (33.21 - 30.43)	32.0 (33.6 - 30.4)	49.3 (96.9 - 5.4)	48.94 (51.10 - 46.72)	7.87 (8.04 - 7.64)	200.4 (285.3 - 67.5)
Dec-05	10.7 (12.1 - 9.4)	18.40 (32.44 - 0.89)	17.9 (32.6 - 0.7)	58.8 (71.1 - 32.6)	20.93 (37.61 - 0.97)	7.97 (8.53 - 7.64)	405.0 (449.6 - 314.9)
Mar-06	11.9 (14.9 - 10.9)	21.78 (33.70 - 11.06)	21.2 (34.0 - 10.0)	41.3 (47.6 - 31.8)	25.14 (38.57 - 12.64)	7.69 (7.90 - 7.49)	NA
Jul-06	21.9 (22.6 - 21.6)	34.11 (36.48 - 29.42)	34.6 (37.3 - 29.3)	59.5 (75.7 - 33.1)	49.39 (53.09 - 43.21)	NA	NA
Oct-06	20.4 (21.2 - 20.1)	35.01 (36.25 - 34.18)	35.7 (37.1 - 34.7)	59.5 (78.4 - 22.4)	49.33 (50.92 - 47.68)	7.83 (8.03 - 7.39)	270.3 (318.0 - 184.0)
Jan-07	14.1 (14.6 - 13.9)	35.24 (36.11 - 34.51)	35.8 (36.8 - 35.0)	59.4 (81.0 - 29.9)	42.96 (43.94 - 41.98)	7.97 (8.10 - 7.68)	253.5 (283.0 - 150.0)
Apr-07	16.1 (17.5 - 15.1)	29.39 (36.15 - 13.87)	28.6 (33.9 - 12.9)	73.4 (107.2 - 52.5)	34.15 (43.99 - 18.30)	7.86 (8.11 - 7.45)	267.4 (292.0 - 231.0)
Jul-07	20.8 (21.6 - 19.9)	35.27 (37.91 - 30.47)	34.8 (37.3 - 30.3)	68.7 (90.7 - 35.1)	47.81 (54.98 - 37.26)	7.75 8.037.34	243.3277.0 206.0
Oct-07	15.3 (16.1 - 14.9)	30.27 (34.39 - 24.18)	28.6 (34.9 - 23.4)	52.3 (70.0 - 16.7)	36.52 (42.62 - 28.68)	7.79 (7.94 - 7.43)	253.4 (258.0 - 243.0)
Jan-08	12.2 (12.8 - 11.7)	29.25 (31.09 - 25.77)	29.1 (31.1 - 25.3)	61.2 (78.6 - 33.4)	33.98 (36.68 - 29.84)	7.60 (7.73 - 7.45)	244.9 (276.0 - 213.0)
Apr-08	13.7 (15.2 - 13.1)	14.96 (29.07 - 0.70)	14.4 (28.9 - 0.5)	65.7 (87.4 - 7.8)	18.00 (34.90 - 0.83)	7.66 (8.04 - 7.31)	240.1 (253.0 - 232.0)
Jul-08	19.3 (22.0 - 13.5)	28.14 (31.51 - 14.97)	28.0 (31.7 - 13.9)	76.8 (88.4 - 68.7)	38.43 (43.11 - 21.70)	7.71 (7.77 - 7.51)	198.6 (254.0 - 107.0)
Oct-08	15.4 (18.5 - 10.6)	23.17 (31.03 - 15.77)	22.7 (31.2 - 14.7)	45.2 (70.7 - 23.6)	29.73 (41.66 - 17.59)	7.48 (7.68 - 7.19)	244.1 (271.0 - 216.0)
Jan-09	9.3	NA	NA	77.1	NA	7.53	268.0
Apr-09	13.7	13.77	12.7	67.6	16.59	7.10	284.0
Jul-09	20.2	NA	26.6	42.2	36.73	7.03	439.0
Oct-09	15.4	24.11	23.5	37.2	30.31	7.24	347.0
Jan-10	8.9	NA	NA	81.0	NA	7.40	183.0
Apr-10	NA	26.84	26.5	75.9	33.19	7.63	384.5
Oct-10	16.8	26.55	26.2	NA	34.43	7.76	192.0

Table 3. The same parameters and conditions as indicated in Table 2, but in samples collected at high tide in surface water.

Campaign	**T(ºC)**	**TDS($g\cdot L^{-1}$)**	**SAL($g\cdot L^{-1}$)**	**DO (% of saturation)**	**κ ($mS\cdot cm^{-1}$)**	**pH**	**ORP(mV)**
Jan-05	5.9 (9.0 – 4.0)	NA	NA	NA	NA	NA	NA
May-05	NA	NA	NA	NA	NA	NA	NA
Sep-05	NA	NA	NA	NA	NA	NA	NA
Dec-05	NA	NA	NA	NA	NA	NA	NA
Mar-06	11.9 (14.0 - 9.8)	6.23 (20.08 - 0.78)	5.7 (19.2 - 0.6)	47.0 (53.2 - 43.6)	7.36 (24.39 - 0.89)	8.06 (8.51 - 7.69)	NA
Jul-06	23.8 (26.2 - 22.6)	25.27 (35.81 - 10.50)	25.0 (36.6 - 9.5)	78.4 (106.8 - 62.1)	37.70 (52.79 - 16.00)	NA	NA
Oct-06	13.9 (22.3 - 19.4)	26.57 (33.62 - 3.19)	26.6 (34.1 - 2.6)	79.6 (80.4 - 60.0)	32.24 (47.15 - 4.39)	7.97 (8.19 - 7.61)	268.0 (320.0 - 192.0)
Jan-07	13.9 (16.7 - 11.3)	26.57 (34.84 - 1.69)	26.6 (35.4 - 1.4)	79.6 (95.9 - 63.3)	32.24 (42.70 - 2.18)	7.97 (8.25 - 7.52)	268.0 (283.0 - 250.0)
Apr-07	17.1 (18.4 - 15.1)	15.78 (32.24 - 2.46)	15.2 (31.2 - 2.0)	74.5 (92.6 - 58.3)	19.01 (36.05 - 3.22)	8.03 (8.35 - 7.72)	268.9 (298.0 - 241.0)
Jul-07	22.8 (23.7 - 21.7)	26.03 (37.92 -4.57)	25.6 (37.4 - 3.9)	88.8 (108.9 - 65.2)	38.27 (58.49 - 6.84)	7.85 (8.43 - 7.17)	248.9 (273.0 - 218.0)
Oct-07	15.1 (17.8 - 13.7)	18.95 (31.71 - 3.98)	17.7 (28.1 - 3.4)	66.7 (90.5 - 56.3)	23.36 (37.71 - 5.28)	7.77 (8.01 - 7.13)	249.3 (256.0 - 233.0)
Jan-08	10.9 1(2.0 - 9.1)	10.24 (27.09 - 0.79)	9.5 (26.7 - 0.6)	74.9 (84.8 - 65.2)	11.70 (31.30 - 0.87)	7.77 (8.14 - 7.51)	235.0 (274.0 - 190.0)
Apr-08	13.9 (16.7 - 13.2)	6.51 (23.61 - 0.37)	6.0 (23.0 - 0.3)	75.4 (85.2 - 61.2)	7.84 (28.34 - 0.45)	7.87 (8.19 - 7.41)	233.0 (245.0 - 208.0)
Jul-08	20.9 (23.5 - 19.3)	17.69 (29.88 - 0.99)	17.3 (29.9 - 0.8)	83.9 (105.9 - 72.6)	24.58 (41.65 - 1.46)	7.93 (8.55 - 7.72)	197.7 (259.0 - 103.0)
Oct-08	16.8 (19.8 - 13.1)	15.19 (24.73 - 7.55)	14.3 (24.2 - 6.6)	40.9 (58.3 - 33.6)	19.64 (33.63 - 10.30)	7.49 (7.73 - 6.85)	240.3 (271.0 - 198.0)
Jan-09	10.1 (11.6 - 8.7)	NA	NA	80.5 (109.0 - 67.2)	NA	7.53 (7.84 - 7.25)	272.5 (286.0 - 252.0)
Apr-09	16.5 (19.1 - 14.3)	5.65 (18.12 - 0.34)	5.1 (17.2 - 0.3)	100.0 (111.4 - 88.8)	7.31 (23.85 - 0.43)	7.40 (7.87 - 6.70)	256.6 (273.0 - 208.6)
Jul-09	21.4 (22.3 - 20.2)	NA	12.4 (25.7 - 1.6)	82.9 (103.2 - 66.4)	18.52 (36.00 - 2.81)	7.42 (7.86 - 7.21)	402.3 (453.0 - 353.0)
Oct-09	14.9 (16.8 - 13.8)	14.67 (29.94 - 3.08)	14.0 (29.9 - 2.6)	44.3 (69.1 - 33.0)	18.21 (37.21 - 3.75)	7.39 (7.72 - 7.11)	336.6 (346.0 - 328.0)
Jan-10	9.5 (10.9 - 8.4)	NA	NA	79.8 (100.3 - 48.8)	NA	7.41 (7.60 - 7.06)	276.9 (351.0 - 166.0)
Apr-10	NA	16.69 (28.03 - 3.30)	15.9 (27.8 - 2.8)	79.8 (85.1 - 72.0)	20.81 (36.96 - 4.06)	7.67 (8.03 - 7.44)	358.5 (428.2 - 215.9)
Oct-10	14.2 (16.2 - 11.9)	11.76 (29.89 - 1.14)	11.1 (29.9 - 0.9)	89.9 (97.8 - 78.9)	14.65 (38.24 - 1.31)	7.79 (8.32 - 7.23)	181.4 (213.2 - 129.2)

Table 4. The same parameters and conditions as indicated in Table 2, but in samples collected at low tide in deep water.

Campaign	**T(ºC)**	**TDS($g \cdot L^{-1}$)**	**SAL($g \cdot L^{-1}$)**	**DO (% of saturation)**	**κ ($mS \cdot cm^{-1}$)**	**pH**	**ORP(mV)**
Jan-05	NA	NA	NA	NA	NA	NA	NA
May-05	17.6 (18.0 - 16.9)	17.69 (24.17 - 9.17)	16.9 (23.6 - 8.3)	60.0 (85.8 - 37.4)	27.33 (37.16 - 14.47)	7.76 (8.04 - 7.29)	241.9 (265.0 - 230.0)
Sep-05	21.5 (21.9 - 21.0)	28.28 (30.64 - 23.58)	28.0 (30.0 - 23.0)	35.5 (70.1 - 3.8)	44.37 (51.79 - 36.27)	7.67 (8.09 - 7.21)	95.9 (184.3 - 60.1)
Dec-05	9.9 (10.4 - 9.4)	9.47 (10.86 - 8.08)	8.4 (9.6 - 7.1)	65.5 (66.7 - 64.3)	10.29 (11.59 - 8.98)	7.84 (8.04 - 7.64)	345.5 (448.9 - 242.1)
Mar-06	11.2 (11.5 - 11.0)	12.95 (21.18 - 4.73)	12.1 (20.2 - 4.0)	46.7 (50.3 - 43.0)	14.62 (23.84 - 5.39)	7.79 (7.82 - 7.75)	NA
Jul-06	23.8 (24.9 - 22.3)	15.59 (36.31 - 2.72)	15.2 (37.1 - 2.3)	55.6 (107.7 - 34.7)	23.48 (55.69 - 3.35)	NA	NA
Oct-06	21.8 (25.1 - 20.3)	14.55 (37.71 - 3.20)	14.1 (38.7 - 2.7)	59.6 (82.3 - 26.5)	21.25 (58.15 - 4.07)	7.47 (7.88 - 6.90)	163.2 (225.0 - 47.0)
Jan-07	13.5 (15.4 - 12.5)	19.91 (36.22 - 3.13)	19.6 (36.9 - 2.6)	56.4 (84.1 - 22.8)	23.78 (42.45 - 3.93)	7.83 (8.06 - 7.64)	268.4 (280.0 - 253.0)
Apr-07	16.9 (19.7 - 15.1)	5.86 (24.55 - 0.92)	5.6 (24.0 - 0.8)	60.7 (104.6 - 42.2)	7.52 (33.96 - 0.18)	7.86 (8.13 - 7.70)	258.8 (288.0 - 238.0)
Jul-07	20.1 (20.8 - 18.8)	7.39 (21.65 - 0.69)	7.7 (25.5 - 0.4)	58.7 (101.0 - 24.7)	10.46 (30.06 - 0.73)	7.60 (8.03 - 7.32)	200.8 (253.0 - 114.0)
Oct-07	15.6 (16.9 - 14.2)	13.40 (29.74 - 4.53)	12.1 (25.6 - 3.9)	59.9 (87.7 - 25.4)	16.52 (35.27 - 5.07)	7.63 (7.93 - 7.23)	260.3 (312.0 - 222.0)
Jan-08	11.0 (14.4. - 8.8)	11.04 (25.91 - 0.53)	10.4 (25.4 - 0.4)	62.0 (82.8 - 37.1)	12.37 (29.28 - 0.56)	7.51 (7.76 - 7.11)	222.4 (264.0 - 199.0)
Apr-08	13.4 (13.9 - 13.1)	18.79 (29.01 - 0.42)	18.4 (28.8 - 0.3)	70.9 (83.7 - 56.2)	22.56 (34.74 - 0.50)	7.27 (8.08 - 6.16)	266.0 (297.0 - 203.0)
Jul-08	19.6 (20.0 - 19.3)	27.09 (31.80 - 17.68)	26.9 (32.0 - 16.7)	77.6 (107.0 - 46.3)	37.40 (43.56 - 24.44)	7.58 (7.61 - 7.55)	242.0 (284.0 - 188.0)
Oct-08	18.3 (18.4 - 18.1)	27.72 (32.01 - 20.87)	27.5 (32.3 - 20.1)	33.6 (47.8 - 26.0)	37.15 (42.76 - 28.01)	6.79 (7.36 - 6.12)	244.3 (301.0 - 203.0)
Jan-09	NA	NA	NA	113.0	NA	7.60 (8.01 - 7.14)	251.0 (272.5 - 239.6)
Apr-09	13.4 (14.3 - 12.5)	18.94 (27.22 - 1.88)	18.4 (26.9 - 1.5)	86.5 (108.5 - 61.1)	22.78 (32.79 - 2.21)	6.80 (7.15 - 6.38)	249.4 (329.0 - 189.0)
Jul-09	20.1 (20.3 - 19.9)	NA	26.7 (29.1 - 25.0)	50.8 (89.8 - 32.0)	36.81 (38.73 - 34.93)	6.98 (7.37 - 6.59)	384.5 (426.0 - 350.0)
Oct-09	15.4 (16.4 - 14.4)	22.77 (27.91 - 16.57)	22.2 (27.7 - 15.6)	42.2 (48.6 - 37.9)	28.61 (34.57 - 21.22)	7.15 (7.32 - 6.88)	359.6 (383.0 - 320.0)
Jan-10	9.5	NA	NA	76.3	NA	6.79	366.0
Apr-10	NA	23.21 (28.89 - 15.90)	23.2 (29.9 - 14.9)	74.7 (83.8 - 58.8)	29.87 (38.84 - 19.88)	7.44 (7.51 - 7.35)	290.6 (376.9 - 160.8)
Oct-10	17.2 (18.1 - 16.5)	27.54 (29.74 - 26.38)	27.3 (29.7 - 26.0)	64.4 (102.4 - 27.2)	36.07 (39.72 - 34.20)	7.62 (7.91 - 7.28)	202.0 (210.9 - 186.9)

Table 5. The same parameters and conditions as indicated in Table 2, but in samples collected at low tide in surface water.

Campaign	T(ºC)	TDS($g\cdot L^{-1}$)	SAL($g\cdot L^{-1}$)	DO (% of saturation)	κ ($mS\cdot cm^{-1}$)	pH	ORP(mV)
Jan-05	6.9 (11.0 - 5.0)	NA	NA	NA	1.60 (5.99 - 0.38)	7.91 (8.10 - 7.52)	NA
May-05	18.3 (20.3 - 16.5)	7.59 (25.54 - 0.54)	7.2 (25.1 - 0.4)	72.5 (97.5 - 31.5)	11.15 (39.30 - 0.00)	7.84 (8.55 - 7.23)	237.8 (406.4 - 142.6)
Sep-05	21.0 (21.7 - 19.6)	19.58 (30.40 - 2.87)	19.3 (30.5 - 2.4)	44.8 (77.5 - 14.6)	30.13 (46.77 - 4.44)	7.79 (8.11 - 7.55)	153.2 (260.0 - 65.4)
Dec-05	10.2 (13.9 - 8.9)	2.16 (5.52 - 0.38)	1.8 (4.7 - 0.3)	61.1 (68.6 - 55.7)	2.35 (6.04 - 0.41)	7.72 (8.12 - 7.25)	316.3 (360.8 - 190.0)
Mar-06	11.2 (15.0 - 9.7)	2.19 (7.33 - 0.27)	1.9 (6.4 - 0.2)	46.4 (50.1 - 42.0)	2.47 (8.32 - 0.29)	7.65 (7.81 - 7.41)	NA
Jul-06	23.1 (24.3 - 21.9)	4.71 (11.48 - 1.24)	4.2 (10.4 - 1.0)	70.9 (101.6 – 41).9	6.66 (16.95 - 1.49)	NA	NA
Oct-06	20.5 (22.1 - 19.2)	7.34 (13.52 - 2.10)	6.6 (12.9 - 1.7)	66.68 (5.4 - 51.9)	8.68 (15.64 - 2.87)	7.54 (7.95 - 6.82)	179.1 (277.0 - 122.0)
Jan-07	12.4 (15.6 - 10.7)	12.58 (27.28 - 2.16)	12.0 (26.9 - 1.8)	65.1 (71.1 - 55.0)	14.34 (31.39 - 2.73)	7.59 (8.15 - 6.62)	267.0 (292.0 - 227.0)
Apr-07	19.0 (21.0 - 17.2)	3.26 (12.15 - 0.66)	2.9 (11.1 - 0.5)	77.9 (99.0 - 69.6)	4.18 (17.20 - 0.15)	7.94 (8.31 - 7.14)	254.4 (277.0 - 233.0)
Jul-07	20.1 (22.1 - 18.4)	4.38 (11.60 - 0.60)	3.9 (10.6 - 0.4)	64.5 (79.5 - 44.7)	6.11 (14.74 - 0.46)	7.72 (7.99 - 7.21)	199.4 (246.0 - 143.0)
Oct-07	15.7 (20.2 - 13.8)	8.08 (14.41 - 3.05)	7.4 (13.4 - 2.5)	61.9 (76.8 - 53.4)	9.94 (17.74 - 3.68)	7.61 (8.00 - 7.19)	223.6 (286.0 - 132.0)
Jan-08	10.4 (14.4 - 8.8)	5.59 (15.07 - 0.50)	5.0 (14.0 - 0.4)	66.7 (79.5 - 57.6)	6.19 (16.85 - 0.53)	7.61 (8.19 - 7.01)	212.3 (276.0 - 148.0)
Apr-08	13.4 (16.3 - 12.6)	3.68 (8.50 - 0.37)	3.2 (7.6 - 0.3)	69.8 (77.2 - 62.8)	4.15 (10.12 - 0.44)	7.57 (7.79 - 7.19)	250.5 (270.0 - 215.0)
Jul-08	20.5 (21.5 - 19.7)	7.31 (19.05 - 1.47)	6.6 (18.2 - 1.2)	81.5 (96.4 - 66.7)	10.27 (26.63 - 2.04)	7.75 (8.13 - 7.53)	217.5 (271.0 - 116.0)
Oct-08	16.5 (18.0 - 13.2)	9.10 (14.83 - 0.12)	8.3 (13.8 - 0.1)	41.3 (44.3 - 39.8)	12.03 (19.78 - 0.15)	7.45 (7.56 - 7.29)	238.5 (266.0 - 213.0)
Jan-09	NA	NA	NA	108.4 (118.0 - 101.9)	NA	7.19 (7.50 - 6.94)	264.2 (275.0 - 250.7)
Apr-09	13.7 (16.9 - 12.3)	5.25 (13.33 - 0.52)	4.7 (12.3 - 0.4)	98.1 (111.2 - 71.9)	6.25 (15.88 - 0.63)	7.11 (7.53 - 6.56)	209.6 (311.0 - 105.4)
Jul-09	20.9 (21.8 - 18.3)	NA	5.7 (18.4 - 1.0)	43.9 (89.4 - 18.8)	8.55 (26.67 - 1.62)	7.09 (7.35 - 6.70)	396.5 (428.0 - 351.0)
Oct-09	15.3 (18.6 - 13.9)	8.59 (22.40 - 2.12)	7.9 (21.7 - 1.7)	56.0 (105.0 - 41.6)	10.68 (28.03 - 2.87)	7.40 (7.77 - 6.62)	350.1 (415.0 - 283.0)
Jan-10	9.3 (11.5 - 8.2)	NA	NA	83.9 (96.1 - 71.4)	NA	7.04 (7.26 - 6.66)	343.2 (363.0 - 335.0)
Apr-10	NA	6.29 (13.83 - 0.95)	5.6 (12.8 - 0.7)	77.7 (91.2 - 66.6)	8.00 (17.95 - 1.23)	7.68 (8.20 - 7.22)	295.7 (382.2 - 196.9)
Oct-10	14.4 (18.1 - 12.4)	6.28 (19.11 - 1.03)	5.7 (18.27 - 0.8)	86.1 (94.8 - 79.6)	7.80 (24.36 - 1.25)	7.74 (8.27 - 7.04)	186.3 (208.3 - 153.3)

In the obvious pattern, the highest conductivity values were found at the mouth of the estuary (*AR*), decreasing from the outermost stations toward the head of the estuary. Similar differences (but less marked) were also observed in deep waters collected at low tide. In this way, it was possible to know the real influence of the tides. The marked differences observed between surface and deep waters confirmed the strong stratification of the water column (low salinity water on the surface and high salinity water at the bottom) previously described (García-Barcina et al., 2006).

Redox potential largely influences the chemical speciation of metals in water and, hence, their fate in estuaries. The values recorded were typical of estuarine waters, with an average value of 262 mV, minimum of 47 mV and maximum of 453 mV. No significant spatial variation was observed between low and high tides, surface and deep waters, or among sampling sites. It is worth noting, however, the high values measured between July 2009 and April 2010, especially in surface waters. In this period redox potentials between 350 and 450 mV were observed along the entire estuary. The concentration of DO in water is another physico-chemical parameter commonly used in quality assessment of environmental areas (Simões et al., 2008). The DO ranged from 3.8 to 118% of saturation, with an average value of 66.5%. Slightly higher values were found in surface than in deep waters. No significant difference was detected, however, between waters collected at low and high tide. The lowest DO values were found in dry seasons, probably due to a decrease in water flow and to decomposition of organic matter from aquatic plants (Whitall et al., 2010). In deep water samples collected at high tide a clear trend was observed, with higher average DO in the outermost sites (*AR*, 71.4%) and lower average values in the innermost sites (*AZ*, 48.1%). As shown in Figure 3b, a similar trend was detected in deep water at low tide (*AR*, 85.2%; *AZ*, 43.7%). In other combinations (surface-high tide and surface-low tide) no remarkable differences in DO among sampling campaigns were identified.

3.1.2. Temporal distribution

To detect increasing or decreasing trends in the time series of the physico-chemical parameters, the Mann-Kendall test was applied separately to deep or surface samples collected at low or high tide. All possible combinations were taken into account. The Mann-Kendall test is a non-parametric method which is able to detect increasing or decreasing trends in time series with a given confidence level. The calculations were made by the Excel template MAKESENS (Mann-Kendall test for trend and Sen´s slope estimates on annual data, Version 1.0, 2002). The statistical basis of this macro is described elsewhere (Ravichandran, 2003; Fdez-Ortiz de Vallejuelo et al., 2010b). The results are shown in Table 6.

The Mann-Kendall test was not applied to the time series corresponding to the deep waters from *GO, UD* and *ZO* at low tide. The weak flow of the river at low tide at those sites did not allow us to distinguish between deep and surface water. Thus, those samples were considered to be surface waters. Similarly, only one sample per campaign was collected at *AR* at low tide and treated as deep water.

The most striking result was a clear decreasing trend in pH in the earlier part of the time series. After July-April 2009 the decreasing trend ended and pH showed an increasing trend thereafter. To illustrate, the time series of pH in deep water from *UD* collected at high tide is shown in Figure 4a.

a)

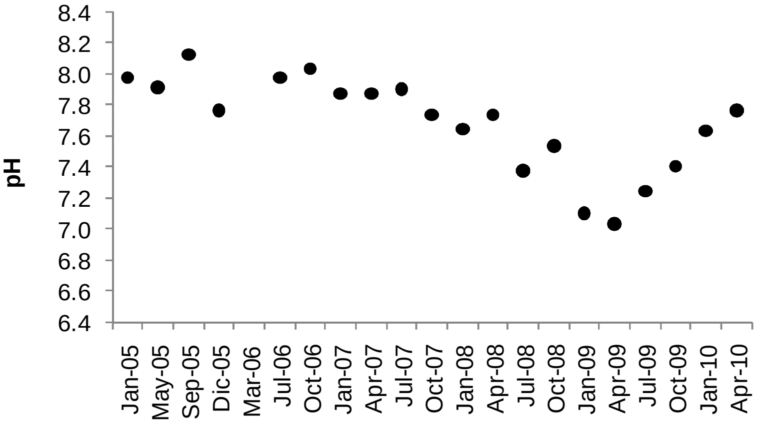

Figure 4. a) pH values measured in deep waters collected in Udondo (*UD*) at high tide between January 2005 and April 2010.

When significant trends were identified in the time series of DO, they were always positive, indicating overall improvement in water quality of the estuary. The constant increase in water oxygenation (see Figure 4b), had been recognised previously for the period 1993-2003 (García-Barcina et al., 2006).

For conductivity two different trends were distinguished: increasing conductivity over time in waters collected at low tide, and decreasing trends in samples collected at high tide. In neither case was the number of sampling sites with significant trends sufficient for drawing general conclusions.

Table 6. Increasing (+) and decreasing (-) trends detected in each sampling site after the application of the Mann-Kendal's test in time series of physico-chemical parameters measured in deep or surface waters collected at high or low tide. 90, 95, 99 and 99.9 express the confidence level at which the trend was detected. "-" means that the test was not performed in those conditions.

	DEEP WATER								**SURFACE WATER**							
Sampling sites	**Low tide**				**High tide**				**Low tide**				**High tide**			
	pH	**DO**	**ORP**	**κ**	**pH**	**DO**	**ORP**	**κ**	**pH**	**DO**	**ORP**	**κ**	**pH**	**DO**	**ORP**	**κ**
AR	-99		+90		-99				-	-	-	-	-99.9			
GO	-	-	-	-	-99				-95				-99	+95		
UD	-	-	-	-	-99.9				-99	+95		+90	-99.9			
GA	-99				-99				-90				-90			
AS	-90	+90		+90	-99.9			-99				+95	-95			
KA	-90				-90	+90		-90				+95				
ZO	-	-	-	-	-99				-95	+95			-99			
AZ						+99			-90				-95			

Table 7. Average metal concentrations reported for different water bodies of the world. The concentrations are in µg·L^{-1} unless otherwise stated.

	Al	As	Cr	Cu	Fe	Mn	Ni	Zn	Ref.
Amvrakiko Gulf (Greece)	-	-	-	0.62	-	-	1.8	3.3	(Scoullos et al., 1994)
Maliakos Gulf (Greece)	-	-	-	0.76	-	-	2.8	1.6	(Scoullos et al., 1987)
Tinto River (Spain)	-	2.9	-	54.7	-	-	-	381	(Elbaz-Poulichet et al., 1999)
Garonne estuary (France)	-	1.5	-	0.80	8.0	-	0.36	1177	(Krapiel et al., 1997)
Tagus estuary (Portugal)	-	51	-	1.6	12.0	-	1.0	15.2	(Elbaz-Poulichet et al., 2001)
Litheos River (Greece)	-	-	-	5.4	-	-	10.0	1.7	(Müller and Foerstner, 1975)
Elbe estuary (German)	-	-	2.5-15.5	3.5-18.0	-	-	3.8-18.6	9.0-194	(Morillo et al., 2005)
Huelva estuary (Spain)	-	7.0-40.0	-	9.0-240	31.0-257	8.0-670	1.0-23.0	38.0-1125	(Morillo et al., 2005)
Thames estuary (England)	-	-	-	10.7	-	-	6.3	29.1	(Power et al., 1999)
Ebro River (Spain)	-	-	-	0.35-5.5	-	-	-	8.2-325	(Ramos et al., 1999)
Mersey estuary (England)	-	-	-	3.2	-	-	-	0.89	(Martino et al., 2002)
Tay estuary (England)	-	-	-	0.05	-	-	-	0.98	(Owens and Balls, 1997)
Gironde estuary (France)	-	-	-	1.4	-	-	-	5.9	(Masson et al., 2006)
Flanders estuary (Belgium)	-	-	-	0.3-14.5	-	-	-	8.0-445	(Bervoets et al., 1994)
Grande de Xubia River (Spain)	-	-	-	2.2	-	-	-	4.6	(Cobelo-Garcia and Prego, 2004)
Lawrence estuary (France)	<2.0	-	-	6.6	4.0	1.5	0.66	0.3	(Rondeau et al., 2005)
Ennore estuary (India)	-	-	-	3330-1090	-	-	-	-	(Padmini and Geetha, 2007)
Seine River (France)	-	-	-	2.0	2.3	-	4.2	13.2	(Chiffoleau et al., 1994)
Mhlathuze estuary (KwaZulu)	160-44412	-	14.0-226	14.0-76.0	-	360-23500	54.0-266	52.0-260	(Mzimela et al., 2003)
Axios River (Greece)	-	-	-	7.0	-	-	-	67.3	(Dassenakis et al., 1997)
Odiel River (Spain)	-	4.7	-	20.4	-	-	-	107.6	(Vicente-Martorell et al., 2009)
Padre Santo Channel (France)	-	3.5	-	11.0	-	-	-	52.4	(Vicente-Martorell et al., 2009)
Po River (France)	6.7	-	-	1197	-	-	-	-	(Alberti et al., 2008)
Gao-ping River (Taiwan)	-	0.63-4.3	0.47-338	0.9-34.3	-	-	3.6-35.8	5.1-354	(Doong et al., 2008)
Vigo Ria (Spain)	-	-	-	0.63	-	-	0.36	1.3	(Santos-Echeandía et al., 2009)
Ferrol Ria (Spain)	-	-	-	0.42-0.60	-	-	-	1.1-1.6	(Cobelo-Garcia et al., 2005)
Nerbioi-Ibaizabal (in µg·kg^{-1})	13.5	22.4	5.4	163	486	37.4	7.0	20.6	This work

b)

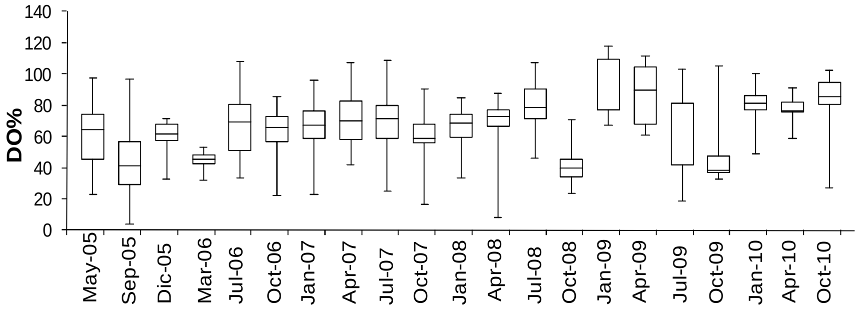

Figure 4 b) Box-Whisker plots of the dissolved oxygen concentration (DO, expressed as % of saturation) measured in each sampling campaign. The box shows the 25th percentile and the 75th percentile, and the whiskers represent the lowest and the highest concentrations, while the line inside the box expresses the average value.

3.2. Trace metal concentration

3.2.1. Comparison with other estuaries

As can be observed in Table 7, the range of trace element concentrations in waters of the estuary of the Nerbioi-Ibaizabal River cannot be considered alarming in comparison with other natural water bodies. In most of the cases, concentrations are comparable to those found in other estuaries of the world. Concentrations of As, Fe and Cu are the exceptions, with moderately higher average values (22, 486 and 162.8 $\mu g \cdot kg^{-1}$ respectively).

3.2.2. Spatial distribution and pollution sources

Basic statistics (average, maximum, minimum and percentiles) of the trace element concentrations measured in surface and deep waters at low and high tide are summarized in Figures 5 and 6. In general, higher concentrations were observed for As, Cu and Fe in deep samples at high tide, and for Cr in surface waters at low tide. The rest of the metal concentrations did not present clear trends. As to geographical distribution, when all data was taken into account, no remarkable difference among sampling sites was found in the case of Al, Cr and Ni. Zn presented slightly higher concentrations in water samples from *GA*, as did Mn in samples from *AZ*. As, Cu and Fe exhibited similar trends, with maximum concentrations in the mouth of the estuary (*AR*), intermediate values in the middle of the estuary (*UD*, *AS* and *GO*) and minimum values in the upper part (*GA*, *KA*, *ZO* and *AZ*).

More specifically, concentrations of the trace elements in deep water collected at high tide (characteristic of ocean water) tended to be homogeneous all over the estuary. Conversely, the trace element content of surface samples collected at low tide (characteristic of the tributary river inputs) significantly changed from site to site, and the distribution was characteristic for each element. Comparing deep water at high tide (DH) vs*.* surface water at low tide (SL) (Figure 6) leads to the following conclusions about metal pollution sources in the estuary:

Cr and Mn: Concentrations of Cr were systematically higher in SL than in DH, indicating a diffuse source of pollution for Cr, entering the downstream reaches of the estuary through all the tributary rivers of the system. The situation is similar for Mn, with the exception of *AZ* (concentration in DH surprisingly much higher than in SL) and *KA* (similar concentrations recorded in DH and SL). Consequently, the Gobela, Galindo and Asua Rivers seem to be local sources of net inputs of Mn to the estuary.

As, Cu and Fe: Concentrations in DH were always higher than in SL, so their sources were not freshwater inputs from the tributaries. It has been reported that the concentration of these elements in sediments of the lower estuary is decreasing with time (Fdez-Ortiz de Vallejuelo et al. 2010b) so, probably, the sediments themselves are acting as secondary sources of As, Cu and Fe to the water.

Ni, Zn: The water samples from DH and SL did not have meaningful differences in the concentrations of these metals. The exceptions were the Galindo River for Ni and Zn, and the Gobela and Asua tributaries for Zn. These rivers are net sources of Ni and Zn to the estuary.

Al: This element was present at slightly higher concentrations in SL from *GA, KA, ZO* and *AZ*, suggesting that Al enters the system (not in large amounts) through the Galindo, Kadagua and Nerbioi-Ibaizabal (but not Gobela) Rivers.

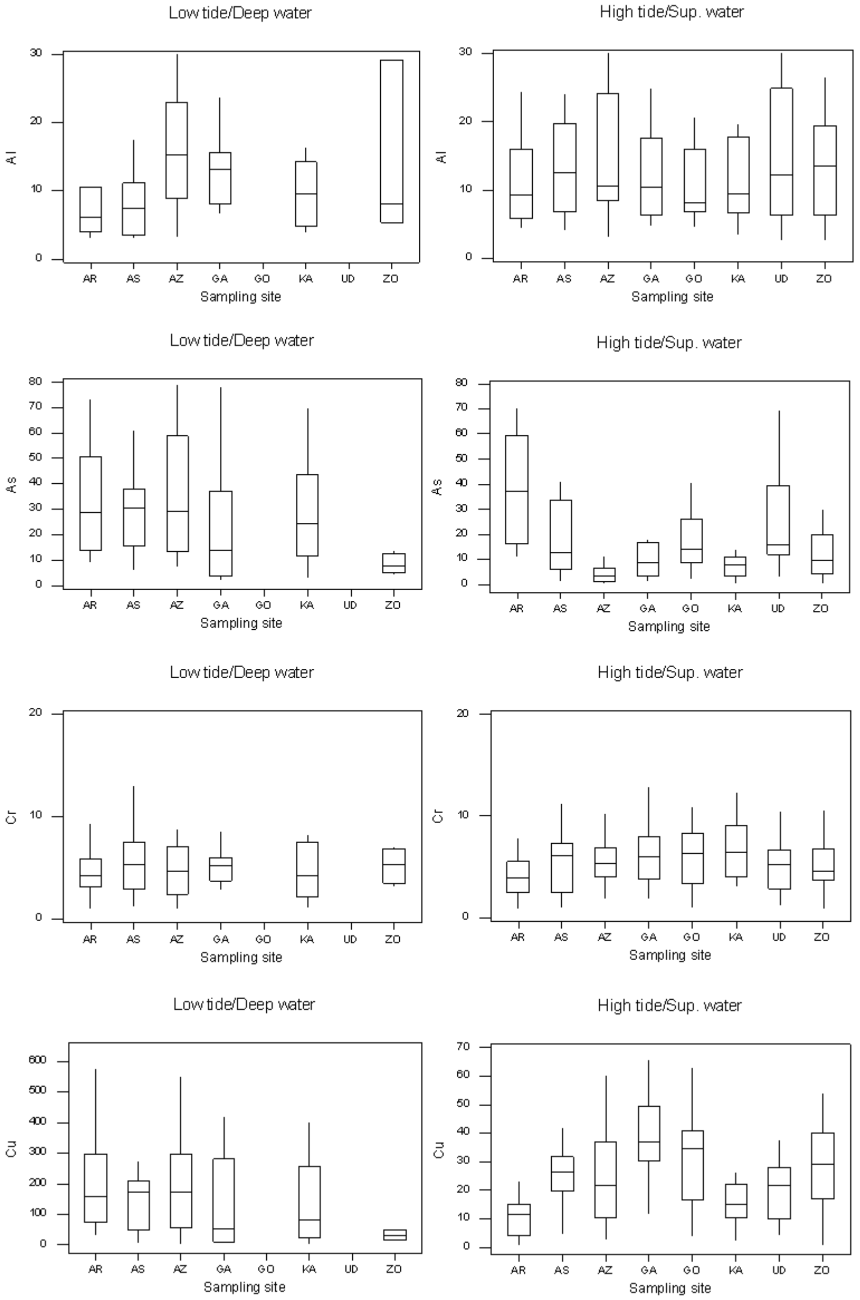

Figure 5. Box-Whisker plots of the trace element concentrations (µg·kg^{-1}) found in deep waters collected at low tide vs. those ones found in surface waters collected at high tide at the eight sampling sites from May 2005 to October 2010. The box shows the 25th percentile and the 75th percentile, and the whiskers represent the lowest and the highest concentrations, while the line inside the box expresses the average value.

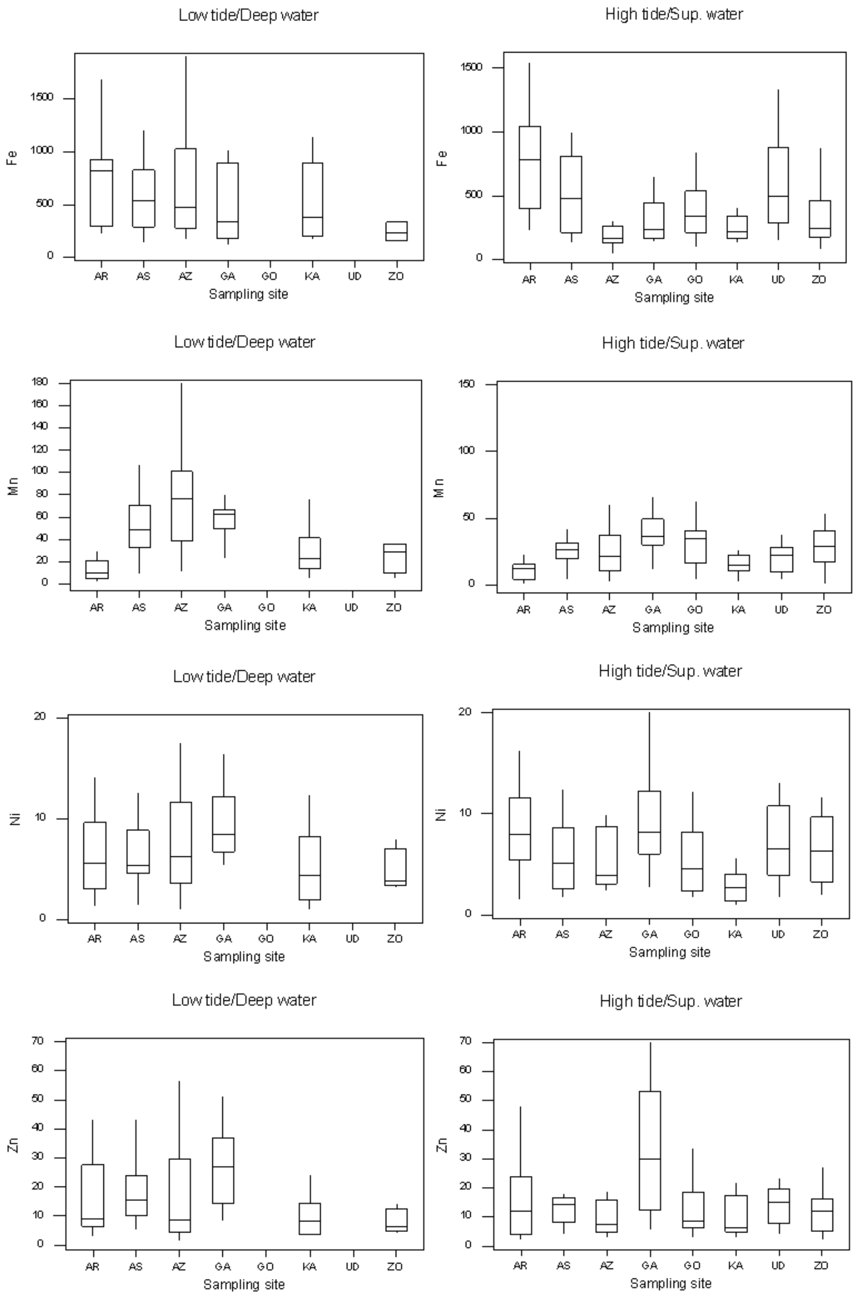

Figure 5. Box-Whisker plots of the trace element concentrations (µg·kg^{-1}) found in deep waters collected at low tide vs. those ones found in surface waters collected at high tide at the eight sampling sites from May 2005 to October 2010. The box shows the 25th percentile and the 75th percentile, and the whiskers represent the lowest and the highest concentrations, while the line inside the box expresses the average value.

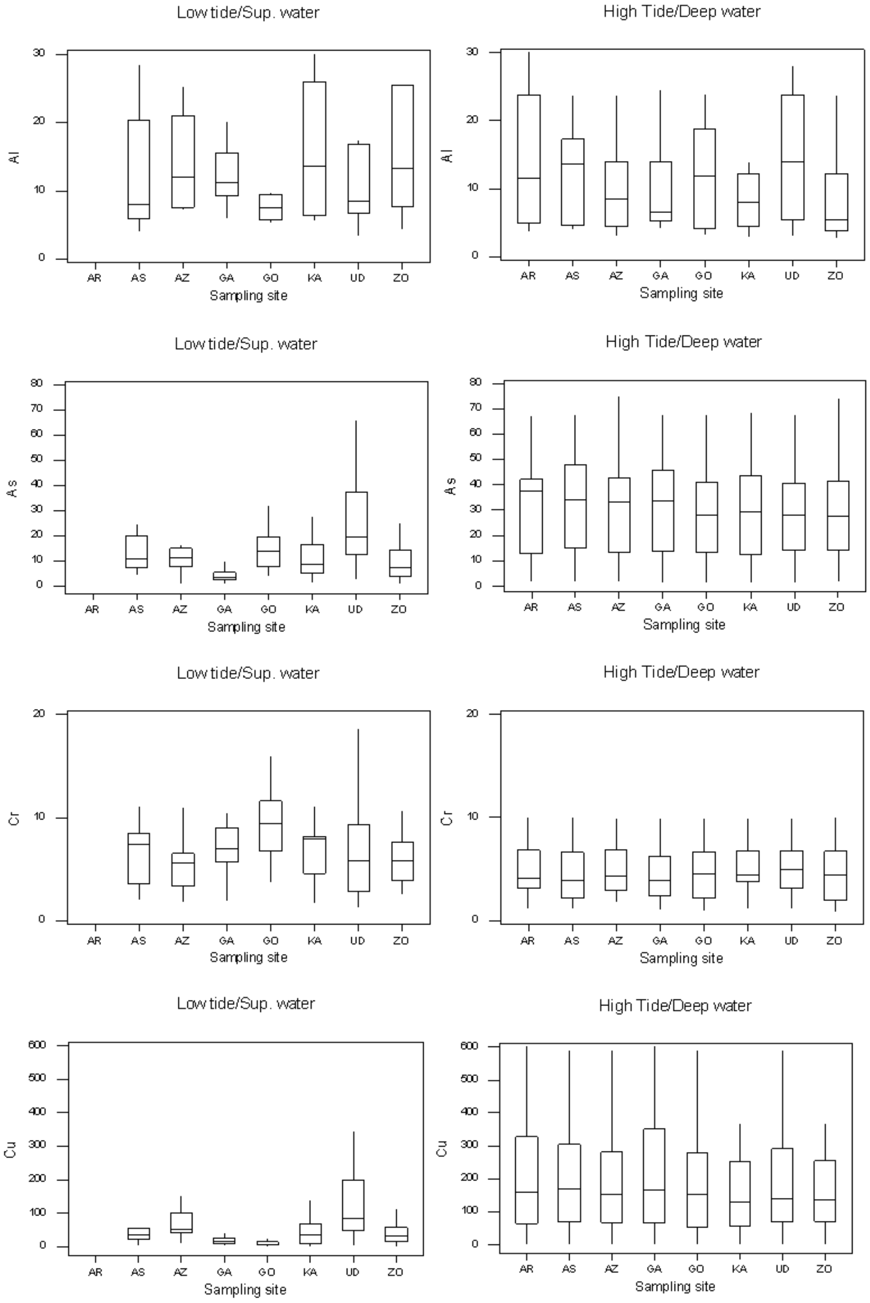

Figure 6. Box-Whisker plots of the trace element concentrations (µg·kg^{-1}) found in surface waters collected at low tide vs. those ones found in deep waters collected at high tide at the eight sampling sites from May 2005 to October 2010. The box shows the 25th percentile and the 75th percentile, and the whiskers represent the lowest and the highest concentrations, while the line inside the box expresses the average value.

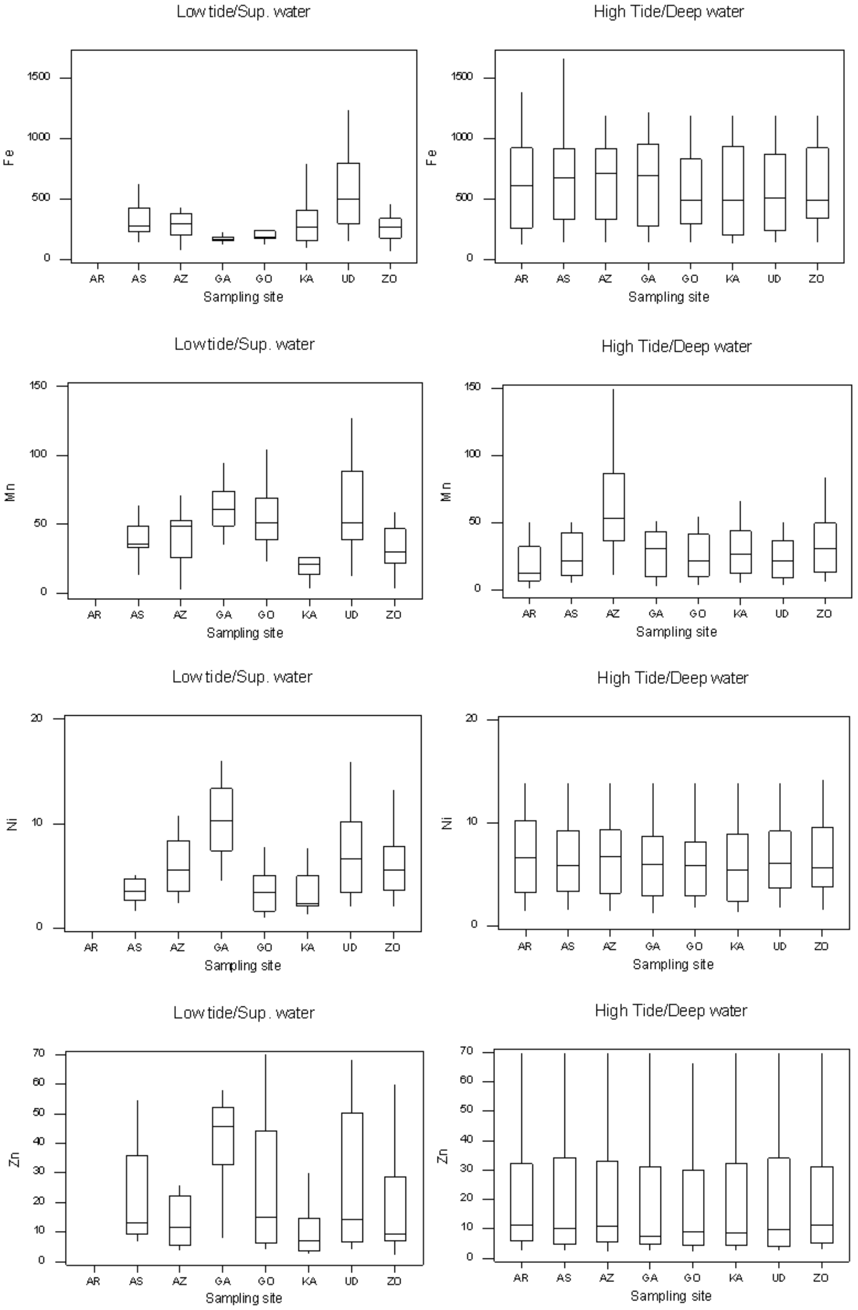

Figure 6. Box-Whisker plots of the trace element concentrations (µg·kg^{-1}) found in surface waters collected at low tide vs. those ones found in deep waters collected at high tide at the eight sampling sites from May 2005 to October 2010. The box shows the 25th percentile and the 75th percentile, and the whiskers represent the lowest and the highest concentrations, while the line inside the box expresses the average value.

3.2.3. Temporal distribution

As a first approach to analysing temporal variations in trace elements, time series of the average metal concentrations were inspected visually. In this way, important trends were detected for As, Cu, Fe and Ni, which presented maximum concentrations in dry seasons (July and October) and minimum values in rainy ones (January and April). Very probably this pattern was a consequence of relatively high temperature and low water flow during dry months. As an example, the average of the Cu concentrations measured in surface waters at high tide is plotted against time in Figure 7, together with the flow data reported for each campaign. The flow was estimated by the sum of the partial flows of the three principal tributaries of the estuary (Ibaizabal, Nerbioi and Kadagua) using data reported by the local administration.[3]

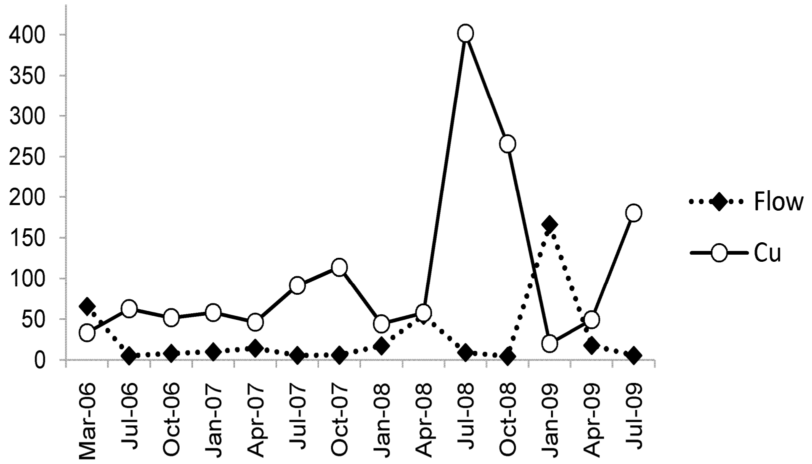

Figure 7. Average concentrations of Cu (µg·kg^{-1}) measured in surface waters collected at high tide, together with the estimated river flow (m^{3}·cm^{-1}) in the sampling campaigns from March 2006 to July 2009.

As in the case of physico-chemical parameters, the Mann-Kendall test was used in order to detect significant increasing or decreasing trends in the time series of the concentrations of trace elements (the time series not analysed for physico-chemical parameters also were not analysed for metals, for the reasons given above).The results are summarized in Table 8. It must be emphasized that 1) when a significant trend was observed it was always positive, and 2) the increasing trend was consistent for nearly all the metals (Al was a clear exception) and for sampling sites in deep water at high tide, that is, in water entering the estuary from the ocean (see Figure 8 as an example).

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[3] http://www.bizkaia.net/Ingurugiroa_Lurraldea/Hidrologia_Ac/Datos_meteo.asp?Idioma=CA&Tem_Codigo=2679

Otherwise, the number of increasing trends decreased in the order: SH>DL>SL, which confirms the likelihood that temporal changes in the characteristics of the ocean water were responsible for the increasing metal concentrations observed in the estuary.

Table 8. Increasing (+) and decreasing (-) trends detected at each sampling site after the application of the Mann-Kendal's test in time series of trace element concentrations measured in deep waters collected at high or low tides and in surface waters collected at high or low tide. 90, 95, 99 and 99.9 express the confidence level at which the trend was detected. "-" means that the test was not performed in those conditions.

	DEEP WATER															
	Low tide								**High tide**							
	AR	**GO**	**UD**	**GA**	**AS**	**KA**	**ZO**	**AZ**	**AR**	**GO**	**UD**	**GA**	**AS**	**KA**	**ZO**	**AZ**
Al	+95	-	-				-						+95		+99	
As	+99	-	-		+95		-	+95	+90	+95	+99	+99	+99	+90	+95	+99
Cr	+99	-	-	+90		+95	-	+99	+95	+99	+95	+99.9	+99	+95	+99	+99
Cu	+99	-	-		+99		-	+95	+90	+95	+99	+95	+99	+95	+99	+95
Fe	+99	-	-		+95		-			+95	+99	+90	+90		+95	+95
Mn	+95	-	-				-		+99	+90	+95	+90	+95			
Ni	+90	-	-		+90	+90	-	+95	+95	+99	+95	+99	+99	+99	+95	+99
Zn	+99	-	-		+90	+90	-		+95	+99.9	+95	+99.9	+99.9	+99	+99.9	+99.9
	SUPERFICIAL WATER															
	Low tide								**High tide**							
	AR	**GO**	**UD**	**GA**	**AS**	**KA**	**ZO**	**AZ**	**AR**	**GO**	**UD**	**GA**	**AS**	**KA**	**ZO**	**AZ**
Al	-								+95							
As	-			+90					+99.9	+95	+99					
Cr	-		+90	+99		+90	+99.9	+95	+95	+90	+99	+99.9	+95	+95	+95	
Cu	-		+99			+90			+99.9		+95					
Fe	-		+95	+99			+90		+95	+90	+90				+95	
Mn	-			+90				+90							+99	
Ni	-		+95			+90			+99		+90		+90			
Zn	-		+99			+95		+95	+99		+99	+90	+95		+95	

3.3. Correlation analysis between trace elements and physico-chemical parameters

All correlation analyses were performed by SPSS, PASW statistic (1200) 18.0 software, using Spearman's non-parametric correlation coefficient (ρ; Gauthier, 2001). First, a correlation analysis with all of the data from the study was carried out. Those cases with "not analysed" or "below detection limit" response in any of the parameters were eliminated from the calculation. The critical value of *r* (N = 545) is 0.11 at a confidence level of 99%, i.e. ρ >0.11 or <-0.11 is significantly different from zero. As could be expected with such a large N, almost all the correlation coefficients calculated fulfilled these conditions.

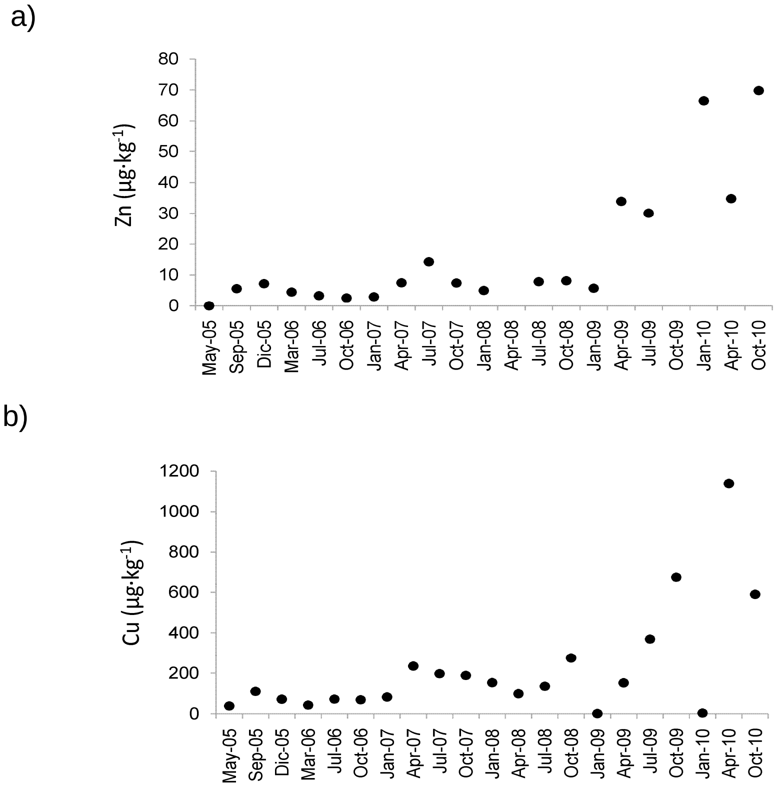


Figure 8. Time series of the a) Zn concentration measured in deep waters of Galindo (*GA*) collected at high tide between May 2005 and October 2010 and b) Cu concentration measured in deep waters of Zorrotza (*ZO*) collected at high tide between May 2005 and October 2010.

Therefore, in order to highlight only the most significant associations, we used a compromise threshold value of ρ = 0.46. The results are shown in Table 9a. Note that all the coefficients above 0.46 were positive. The strongest relations observed were those ones among As, Cu and Fe, with ρ = 0.85–0.90. These three trace elements also were correlated with Ni (ρ ~ 0.5) and with conductivity (As, ρ = 0.51; Cu, ρ = 0.60 and Fe, ρ = 0.55). Concentrations of Ni and Zn also were positively correlated (ρ = 0.58).

Second, the samples were divided into four groups, i.e. samples collected at low tide (L), at high tide (H), in surface water (S) and in bottom water (D). The number of samples was approximately half of the initial data (N = 227–318), so that a threshold value of ρ = 0.54 was chosen to investigate the strength of the relationships among variables. Correlations for samples collected at high tide (H) and those from the bottom (D) were similar. In both cases all the significant correlations were positive, and again, the strongest were among As, Cu, Fe and

Ni, as one group, and between Ni and Zn, as another. For these samples, no strong association was found between the trace elements and conductivity (Table 9b).

Table 9. Correlation matrix obtained using: a) all the data; b) those samples collected at high tide and c) those samples collected in the surface. Correlations significantly different from zero at a 99% confidence level are marked in bold, and the most significant ones (a) >0.46; b) and c) >0.54) are underlined.

a)

	Al	**As**	**Cr**	**Cu**	**Fe**	**Mn**	**Ni**	**Zn**	**T**	**DO**	k	pH	**ORP**
Al	1.00												
As	**-0.23**	1.00											
Cr	**0.39**	0.04	1.00										
Cu	**-0.18**	**0.90**	-0.04	1.00									
Fe	-0.10	**0.85**	-0.02	**0.90**	1.00								
Mn	0.06	0.07	**0.34**	0.03	0.07	1.00							
Ni	**0.17**	**0.47**	**0.26**	**0.50**	**0.50**	**0.34**	1.00						
Zn	**0.44**	0.10	**0.42**	**0.15**	**0.16**	**0.42**	**0.58**	1.00					
T	**-0.18**	**0.23**	**-0.24**	**0.27**	**0.27**	0.07	**0.18**	-0.07	1.00				
DO	**0.17**	**-0.21**	0.08	**-0.25**	**-0.16**	**-0.15**	0.01	**0.15**	-0.05	1.00			
k	**-0.25**	**0.51**	**-0.39**	**0.60**	**0.55**	**-0.28**	0.11	**-0.14**	**0.35**	**-0.16**	1.00		
pH	-0.09	**-0.23**	**-0.19**	**-0.18**	**-0.18**	**-0.41**	**-0.37**	**-0.42**	-0.01	**0.22**	-0.05	1.00	
ORP	0.06	**0.15**	0.10	**0.16**	**0.12**	-0.11	0.05	0.04	**-0.13**	-0.10	0.03	**-0.21**	1.00

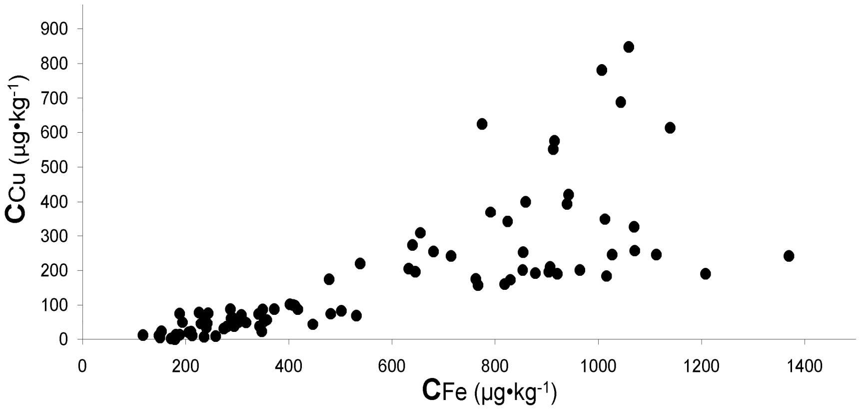
b)

	Al	**As**	**Cr**	**Cu**	**Fe**	**Mn**	**Ni**	**Zn**	**T**	**DO**	k	**pH**	**ORP**
Al	1.00												
As	**-0.22**	1.00											
Cr	**0.45**	**0.17**	1.00										
Cu	-0.18	**0.93**	0.10	1.00									
Fe	-0.11	**0.86**	0.07	**0.92**	1.00								
Mn	0.07	0.12	**0.43**	0.11	0.14	1.00							
Ni	0.13	**0.59**	**0.35**	**0.59**	**0.59**	**0.32**	1.00						
Zn	**0.44**	**0.25**	**0.49**	**0.28**	**0.27**	**0.51**	**0.58**	1.00					
T	**-0.28**	**0.28**	**-0.24**	**0.35**	**0.35**	-0.03	**0.20**	-0.07	1.00				
DO	**0.26**	**-0.24**	0.12	**-0.25**	**-0.18**	-0.07	0.07	**0.22**	0.01	1.00			
k	**-0.32**	**0.32**	**-0.47**	**0.40**	**0.41**	**-0.24**	0.01	-0.14	**0.49**	**-0.21**	1.00		
pH	-0.10	**-0.34**	**-0.27**	**-0.25**	**-0.24**	**-0.47**	**-0.33**	**-0.44**	0.06	**0.20**	0.01	1.00	
ORP	0.02	**0.26**	0.09	**0.22**	**0.19**	-0.04	**0.18**	0.07	0.00	-0.16	-0.08	**-0.27**	1.00

c)

	Al	**As**	**Cr**	**Cu**	**Fe**	**Mn**	**Ni**	**Zn**	**T**	**DO**	k	**pH**	**ORP**
Al	1.00												
As	**-0.32**	1.00											
Cr	**0.29**	0.03	1.00										
Cu	-0.17	**0.82**	-0.12	1.00									
Fe	-0.08	**0.79**	-0.02	**0.82**	1.00								
Mn	-0.11	0.01	**0.30**	-0.07	-0.03	1.00							
Ni	0.11	**0.26**	0.11	**0.38**	**0.36**	**0.28**	1.00						
Zn	**0.40**	-0.05	**0.28**	0.03	0.09	**0.35**	**0.52**	1.00					
T	**-0.21**	**0.29**	-0.15	**0.27**	**0.29**	0.12	**0.18**	-0.08	1.00				
DO	**0.24**	-0.14	0.03	**-0.21**	-0.09	-0.09	0.02	0.13	0.06	1.00			
k	-0.15	**0.60**	**-0.33**	**0.73**	**0.70**	**-0.27**	**0.24**	-0.06	**0.35**	-0.07	1.00		
pH	-0.05	-0.13	-0.15	-0.16	**-0.17**	**-0.33**	**-0.34**	**-0.47**	-0.07	0.15	-0.14	1.00	
ORP	0.10	0.01	0.06	0.08	0.06	**-0.20**	-0.06	0.10	-0.09	-0.08	0.11	**-0.23**	1.00

The results of the correlation analysis done with the samples collected at low tide (L) and the samples from the surface (S) also were similar (Table 9c). For these samples, apart from the relations observed among As, Cu and Fe (ρ = 0.85–0.92), there were again significant positive correlations between As, Cu, Fe and conductivity. As an example of the positive correlation, in Figure 9 Fe is plotted against Cu for deep water, low tide samples.



Figute 9. Fe and Cu concentrations in deep water samples collected at low tide from May 2005 to October 2010.

We observe that the correlation patterns obtained for deep and high tide samples, on the one hand, and those for surface and low tide samples, on the other, are similar. Note that deep water at high tide is characteristic of the ocean and surface water at low tide is fluvial. These two groups, SL and DH, were independently analysed. In this case, ρ = 0.63 was chosen as the threshold value, due to the decrease in the amount of samples (N = 91–169). As expected, the results obtained in SL did not differ from those obtained for S and L independently, and the same finding applies to the DH group.

4. Conclusions

This study confirms that the Nerbioi-Ibaizabal estuary is a tidal and stratified estuary. Although the tide was not a limiting factor for the amount of dissolved oxygen detected in water, the depth at which the sample was collected showed a clear influence on this variable. The evolution in time of the dissolved oxygen measured in water suggests that the ecological quality of the Nerbioi-Ibaizabal estuary is improving significantly. Data also confirms that the influx of seawater, the biological activity, the atmospheric deposition, and the tributary rivers should be considered as inputs of oxygen in the estuary. Unexpectedly, a significant and general decrease in water pH was detected until January 2009, a trend that became positive after that date. In addition, flow discharge seems to play an important role in the concentrations of some of the trace elements considered. As, Cu, Fe and Ni presented an important dilution effect, which maximum values in dry seasons and minimum values in wet ones. This fact may condition a possible seasonal trend in the concentration of these elements.

In general terms, the situation of the waters of the estuary concerning metal pollution is not alarming if compared to other similar environments in the world. Three possible metal pollution sources were identified, however, in the estuary: i) the tributary rivers (except Kadagua); ii) the ocean, and iii) the sediments themselves, which are able to accumulate contaminants for long time periods. In fact, several findings in this work seem to point towards the same direction: first, the concentration in water of nearly all trace elements considered increases with time in the period investigated; second, this fact is more pronounced in deep samples collected at high tide, where strong positive correlations were found between As, Cu, Fe, Ni and conductivity; third, a general decreasing trend in water pH was found within the studied period. Furthermore, we have seen in previous investigations (Fdez-Ortiz de Vallejuelo et al., 2010b) that the metal content in sediments of the lower part of the estuary is decreasing with time. As a hypothesis, it could be suggested that sediments are acting as secondary source of metal pollution (at least As, Fe, Cu and Ni) towards the estuary water, a fact which could be connected with the decreasing trend observed in water pH. Acceptance or rejection of this hypothesis, however, requires further investigation, and we are currently working in that direction.

Finally, we want to highlight the design itself of the monitoring exercise carried out in this work. On the one hand, collecting deep and surface water at high and low tide is laborious and time consuming, but it has been an essential point to draw the conclusions compiled in this work. Furthermore, collecting samples every three months allows us to investigate possible seasonal trends, which is not possible when, as most usually occurs, annual campaigns are performed. The investigation of seasonal trends, however, requires long time series if definitive conclusions are to be obtained.

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Long term seasonal monitoring of metal pollution in sediments from the estuary of the Nerbioi-ibaizabal River

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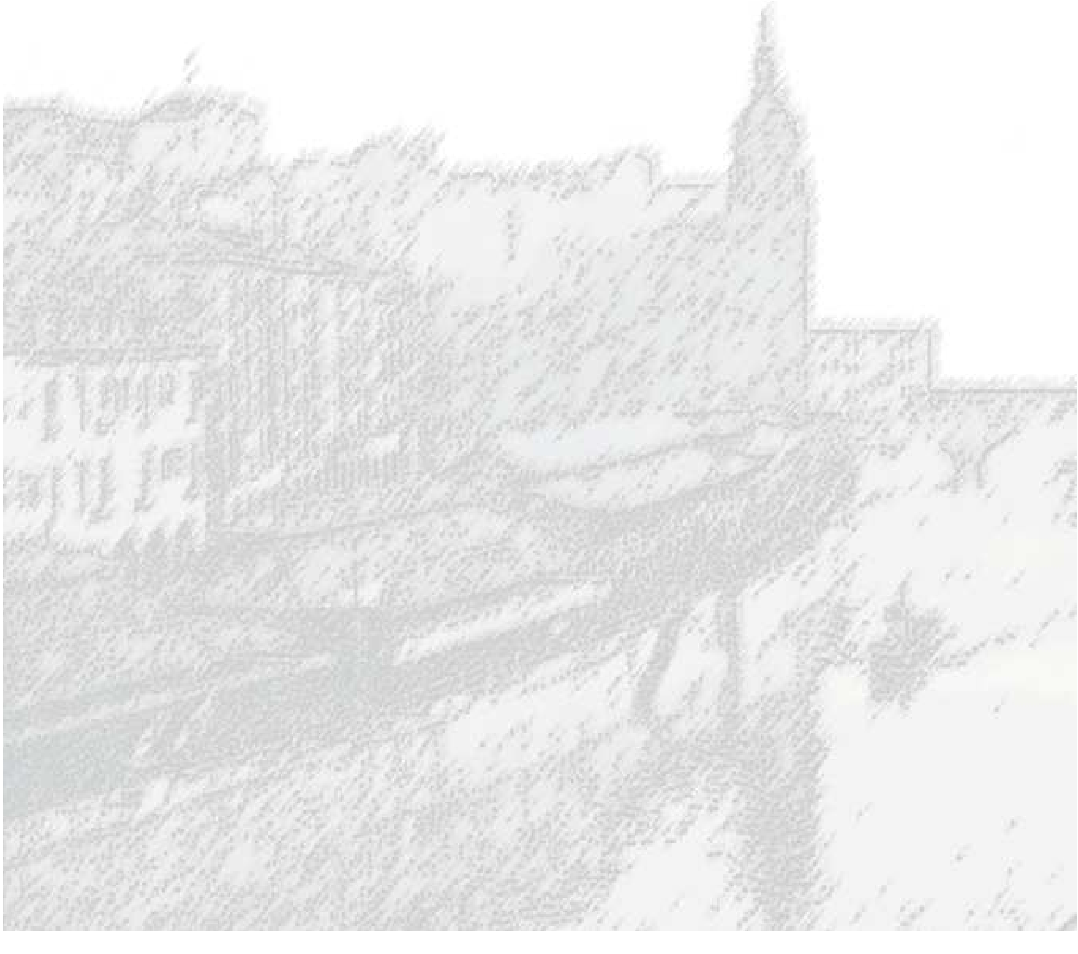

Long term seasonal monitoring of metal pollution in sediments from the estuary of the Nerbioi-ibaizabal river

Abstract

Sediments are considered a fundamental compartment of aquatic ecosystems. A correct management of sediments, therefore, is essential for the adequate use and management of marine systems as estuaries. Pollution by metals is of concern due to their long persistency in sediments and the toxic effects that they may cause in the abundant marine organisms that grow up in these environments. The spatial and temporal distribution of metal pollution was investigated in sediments along the *Nerbioi-Ibaizabal* estuary (Bilbao, Basque Country). Samples were collected at eight different sites of the estuary every three months between January 2005 and October 2010. The conductivity, pH and the content of carbonate, total organic carbon, fulvic acids and humic acids, together with the concentration of 14 selected elements were measured in the samples. Graphical analysis of data, geoaccumulation indexes and Sediment Quality Guidelines (SQGs) were used to analyse the results. Point sources of pollution were detected over a background of contamination of diffuse origin. Although in general terms a clear decreasing trend with time was observed in the concentration of the most toxic metals, two points of concern were detected in the estuary: the low part of the Gobela tributary river and the surroundings of a waste water treatment plant operating in the area. The concentrations of As, Cd, Cu, Ni, Pb and Zn in most of the sediments collected in those sites are well above the background values previously defined for the basin and may represent nowadays a potential toxicological threat to living organisms.

Keywords: Nerbioi-Ibaizabal, Bilbao, trace elements, estuaries, sediments, pollution monitoring, risk assessment.

1. Introduction

The European Water Framework Directive[1] was introduced in 1999 as the most ambitious and thorough environmental legislation regarding the water quality in the European Union (EU). Its principal aim is to achieve the good quality status by 2015 for all water bodies of the European member states. The WFD differences three monitoring types: surveillance, operational, and investigative monitoring.[1] All these monitoring programs, however, are principally aimed to water analysis, while few specifications are intended for other important compartments of the water bodies, as sediments or biota.[2] Moreover, while strong water quality objectives have been designed by the directive, in sediments and biota the main objective is just to avoid an increase in pollutant concentration.[3]

A list of 33 chemicals is defined in the WFD as priority substances due to their potential toxicity. Most of them are organic contaminants such as pesticides or organochlorine compounds and although metals and their compounds are also generally included only four metals (Sn, Hg, Cd and As) and one organometallic compound (tributyltin) are specifically mentioned.[1] More metals have been largely investigated, however, due to their well recognised toxic behaviour, such as, Zn, Cu, Ni, Cr and Pb.[4] All these trace elements are included in the list of key pollutants of the European Pollutant Release and Transfer Register (E-PRTR, http://prtr.ec.europa.eu/Home.aspx).

The implementation of the WFD directive is of special importance in transitional water bodies, e.g., estuaries, due to their ecological and economical importance.[5] The main inputs of trace metals to estuaries are industrial and urban sewage waters, land run-off, and atmospheric deposition. When trace elements enter the estuary, they are adsorbed on suspended particulate matter and finally accumulated in the upper sediment, principally in the fine-grained fraction.[6] Trace elements stored in sediments normally act as risky contaminants, owing mainly to their toxicity, long residence time and bioavailability. In addition, a change in the physico-chemical properties of the underlying water may remobilize metals to the water column, increasing their availability to living organisms. Thus, the simple monitoring of water is clearly not enough for an adequate and complete environmental risk assessment in estuaries. Long-term monitoring of sediments provides us with important complementary information to investigate spatial and temporal trends in trace metal pollution.[7]

Approaches based exclusively on chemical analysis, however, do not take into account the specific geochemical characteristics of each estuary, or the real toxicological impact of contaminants in living organisms. The use of a variety of numerical indexes has allowed an adequate comparison among different aquatic environments. The enrichment factor (EF)[8], the geoaccumulation index (I_{geo})[9] and the contamination factor (CF)[10] have been commonly used with this purpose. They give us the possibility to differentiate between the natural and anthropogenic fraction of the trace element contained in the sediment, using the background value defined for the area as a reference. Unfortunately these indexes are not able to estimate potential toxic effects of metals in living organisms. To solve this problem, different Sediment Quality Guidelines (SQGs), connecting toxicity to trace element concentration, have been developed.[11] Although AETs (apparent effects threshold), PELs (probable effects level) or TELs (threshold effects level) have been frequently used, ERMs (effects range-median) and ERLs (effect range-low) are the most popular ones.[12] Since the SQGs only consider the toxicity of individual chemicals, the use of mean SQG quotients (mSQGq) has been proposed to take into account simultaneously the effect of a variety of pollutants present in the sediment.[13]

In this work we present the results of a long-term monitoring of sediments carried out in the estuary of the *Nerbioi-Ibaizabal* River (Bilbao, Basque Country). This area has been subjected to a considerable industrial and mining activity throughout its history[14], and it is located in one of the most important urban areas of the Bay of Biscay. The purpose of this study is to investigate the space and time distribution of trace metals in the estuary during a long-period seasonal monitoring program of 6 years (2005-2010), and to estimate the potential toxicological risk associated to the presence of these pollutants in sediments. The results from the 2005-2007 period were already presented in a previous article.[15] Now we report new data from the 2008-2010 period and analyse the whole dataset (2005-2010) to draw general conclusions concerning metal pollution and derived toxicological implications. Different tools have been combined to fulfil the objective: i) the analysis of time series of physico-chemical properties of the sediment (pH, conductivity, and carbonate, total organic carbon, humic acid and fulvic acid contents), and trace metal concentration, ii) the calculation and interpretation of geoaccumulation indexes, and iii) the use of SQGs to assess the toxicological impact due to the presence of trace metals in the sediments. The results have allowed us to identify potential risky areas, which facilitates a future sustainable management of the estuary.

2. Materials and methods

2.1. Study area and sampling design

The estuary of the *Nerbioi-Ibaizabal* River (Metropolitan Bilbao, Basque Country) is located in the continental shelf of the Cantabrian Sea, in the north coast of the Iberian Peninsula (Figure 1). Its principal freshwater input comes from the *Nerbioi* and *Ibaizabal* Rivers (68%), but it also receives the freshwater input of four tributaries (*Kadagua* 27%, *Galindo* 4%, *Asua* 0.7% and *Gobela* 0.3%). An important mining and industrial activity starting at the end of the XIX^th^ century led to the environmental collapse of the system by the middle of the XX^th^ century.[14] The situation of the estuary has improved significantly since 1990, mainly due to the closure of most industries, mine workings, and to the implementation of an ambitious plan for the integral recovery of the estuary ("Strategy for the integral recovery of the estuary of Bilbao").[14, 16] The estuary still suffers, however, the pressure coming from a million people living around it.[17] Several works have investigated trace element pollution in the estuary with different objectives and using a variety of approaches.[15, 16, 18-38]

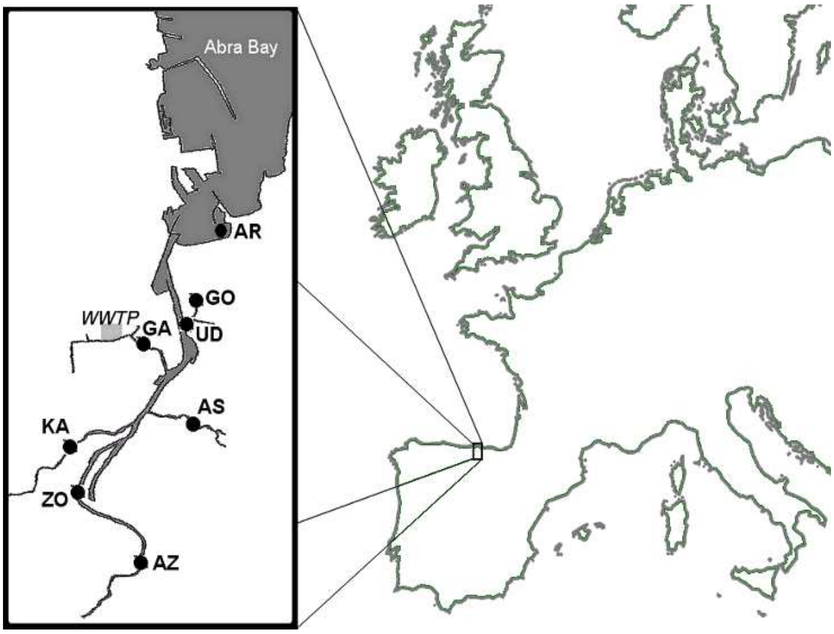

Figure 1. Estuary of the Nerbioi-Ibaizabal River and location of the eight sampling sites: 1*AR* (Arriluze), 2*GO* (Gobela), 3*UD* (Udondo), 4*GA* (Galindo), 5*AS* (Asua), 6*KA* (Kadagua), 7*ZO* (Zorrotza) and 8*AZ* (Alde Zaharra). WWTP: Waste water Treatment Plant.

Taking into account the previous works mentioned above, eight sampling sites were conscientiously chosen: two of them are located in the main channel (*ZO* and *AZ*), one in the mouth of the estuary (*AR*), another one in a semi-closed dock (*UD*), and the rest are situated in the tidal part of the principal tributaries: Gobela (*GO*), Asua (*AS)*, Kadagua (*KA)* and Galindo *(GA)*. This last site is about 500 m downstream the effluent-output of the most important sewage plant of the area (see Figure 1). A total of 184 sediment samples were collected at low tide in those sites since January 2005 to October 2010, approximately every three months. Details on the sampling procedure are given elsewhere.[15]

2.2. Analytical methods

All the lab ware used during sampling and analysis was cleaned with water and soap, thoroughly rinsed with Milli-Q water ($\kappa < 0.05\ \mu S \cdot cm^{-1}$, Millipore, Billerica, MA, USA) and immersed in a 10% HNO_3 bath for 24 h. Finally, all the material was soaked again with Milli-Q water and dried in an oven at 50 °C. The concentration of Al, As, Cd, Co, Cr, Cu, Fe, Mg, Mn, Ni, Pb, Sn, V and Zn was measured in all the sediments. Sample pretreatment and analysis are also described in detail elsewhere.[15, 39] The analysis of the NIST 1640 certified reference material (estuary sediment, National Institute of Standards and Technology, Gaithersburg, USA) confirmed the accuracy of the method, and the next detection limits (in mg of element per kg of sediment) were estimated for the trace elements considered: Al 2.2, As 0.2, Cd 1.0, Co 0.7, Cr 0.2, Cu 1.5, Fe 1.6, Mg 0.8, Mn 1.2, Ni 0.3, Pb 0.8, Sn 1.4, V 0.5 and Zn 1.2. The analytical reproducibility of the method was estimated by replicate (n= 5) analysis of a sample collected in *ZO* in October of 2009. The sampling reproducibility was also calculated by analysis of six different sediment samples collected in rectangles of approximately 6 m^2 (2m×3m) also in *ZO* sampling point during the campaign of April 2010.The analytical and sampling reproducibility estimated for each element, expressed as relative standard deviation were as follows : Al 3 and 14, As 5 and 23, Cd 4 and 19, Co 2 and 5, Cr 4 and 16, Cu 1 and 11, Fe 4 and 11, Mg 0.5 and 4, Mn 2 and 7, Ni 2 and 3, Pb 2 and 12, Sn 5 and 8, V 2 and 7 and Zn 2 and 13 respectively.

The pH, conductivity (κ), and content of carbonates, total organic carbon (TOC), humic acids (HA) and fulvic acids (FA) were measured in the fine fraction (< 63 µm), only in sediments collected between March 2006 and October 2008. The pH and conductivity were measured in a 1/5 sediment/MilliQ water suspension after equilibration for 18h and 0.5 h respectively.[40] The total organic carbon contained in the dried sediment was determined by redox titration following the procedure proposed by Page et al. 1985.[41] An acid-base back-titration of a suspension of 1g of sample in an excess of 0.25M H_2SO_4 was used to determine the carbonate content.[42] Finally, the analysis of the humic and fulvic acid content was performed by UV absorption spectroscopy (Jasco v-670, Tokio) following the procedure optimised in our laboratory. [43] Briefly, about 0.1 g of sediment was mixed with 15 mL of 1M NaOH (Puriss, %98, Fluka), which was previously deoxygenated under a N_2 atmosphere. Focused ultrasound energy (HD 2070 Sonoplus Ultrasonic Homogenizer equipped with a MS 73 titanium probe, Bandelin, Germany) was applied to the suspension for 29 min at 95% of the total power. The leachate containing the FA and HA was separated by centrifugation from the residual solid, and then acidified with about 5 mL of 6M HCl (Tracepur, %36, Merck). In acidic media humic acids precipitate while fulvic acids remain in solution. Both phases were separated again by centrifugation, and the concentration of FA was spectrofotometrically measured at 271 nm in the clear acid solution. The solid was redissolved in a NH_3/NH_4^+ buffer solution and the HA concentration was also spectrofotometrically measured at 273 nm.

3. Results and discussion

3.1. Spatial distribution of physico-chemical parameters

The physico-chemical properties measured in the sediments are summarized in the form of Box-Whisker plots in Figure 2. Figure 2a illustrates their spatial distribution in the estuary. As it could be expected the conductivity decreases with increasing distance from the mouth of the estuary. The average pH of the sediments varied from 6.7 to 7.85, and no significant difference was observed among sampling sites. The carbonate average content ranges from 2.9% to 7.6% and is significantly higher in *KA* than in the rest of the stations. No significant differences were observed in the TOC of sediments from different stations. The dispersion of data, however, was higher in *AZ* and *KA* than in the rest of sites. Humic and fulvic substances play an important role in sediments regarding the bioavailability of pollutants, due to their ability to adsorb ions at low pH.[44] The average HA values found in the sampling sites range from 510 $mg{\cdot}kg^{-1}$ to 1650 $mg{\cdot}kg^{-1}$. For FA, they go from 5400 $mg{\cdot}kg^{-1}$ to 6900 $mg{\cdot}kg^{-1}$. A slightly increasing trend in HA concentration was observed with increasing

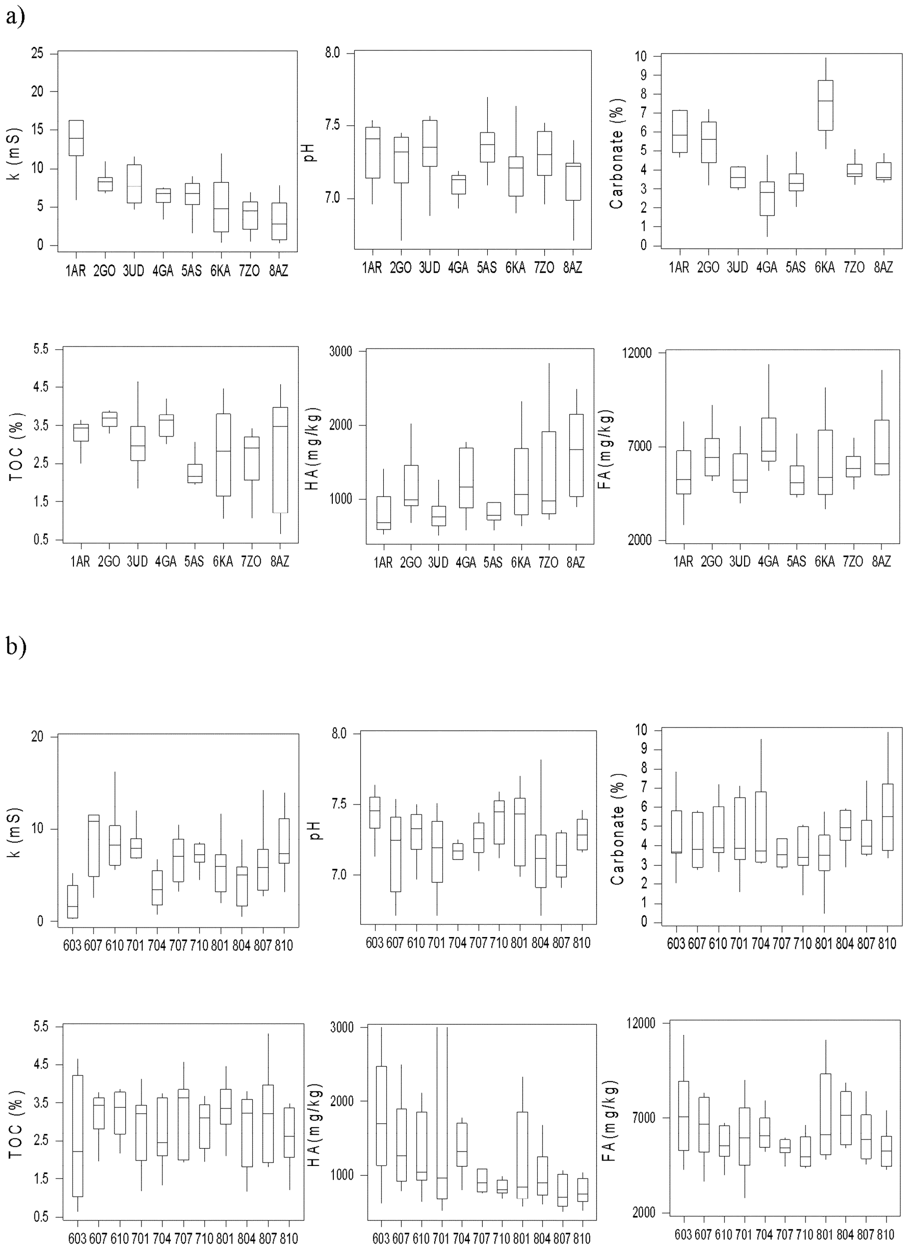

Figure 2. Box-Whisker plots of the conductivity, pH, carbonate (CO_3^{2-}), total organic carbon (TOC), humic acids (HA) and fulvic acids (FA) measured in sediments collected between March 2006 and October 2008 a) at each sampling site; b) in each sampling campaign. The first number in the X axis labels of Figure 2b indicates the sampling year, and the second and third numbers the month. The box shows the 25th percentile and the 75th percentile, and the whiskers represent the lowest and the highest values, while the line inside the box expresses the average value.

distance from the mouth of the estuary. The average FA concentrations in sediments from different sites, however, keep quite constant all along the estuary.

3.2. Temporal distribution of physico-chemical parameters

The time series of the measured parameters can be seen in Figure 2b. Only in the case of conductivity was observed a clear seasonal trend, with maximum conductivity values in summer-autumn (lower rainfall) and minimum ones in spring-winter (higher rainfall). The concentration of HA in the sediments also seems to slightly decay along the time series, but this trend is not marked enough to extract definitive conclusions. To go further in detail, the Mann-Kendall test was applied specifically in each sampling site for each measured parameter. This test is a non-parametric method which is able to detect increasing or decreasing trends in time series with a given confidence level. The calculations were made by the Excel template MAKESENS (freely available in www.emep.int/assessment/MAKESENS_1_0.xls). The HA content presented a decreasing profile in time, detectable at a 95% confidence level, in *ZO, AS* and *GA* sampling sites, and at a 99% confidence level in the *GO* sampling sites. The carbonate content showed a slight positive pattern (detectable at a 90% of confidence) only in the *AS* sampling site.

3.3. Spatial distribution of trace metals and possible sources of pollution

Basic statistics (average, maximum, minimum and percentiles) of the trace element concentrations measured in the sediments collected from January 2005 to October 2010 are summarized in Figure 3. Correlation analysis of data was also done. The Spearman's rank correlation coefficient (or Spearman's rho), which has been frequently used in pollution monitoring studies[45, 46] was calculated because it is more reliable than the classical Pearson coefficient when dealing with not normally distributed data. The calculation of the coefficients was performed by SPSS, PASW statistic (1200) 18.0 software, and the correlations obtained are shown in Table 1. If the 184 samples analysed are considered, a Spearman rho (in absolute terms) higher than 0.19 indicates that the correlation coefficient is significantly different from zero at a confidence level of 99%. For practical reasons, a threshold value of 0.64 was arbitrarily considered to be indicative of a strong connection between two variables.

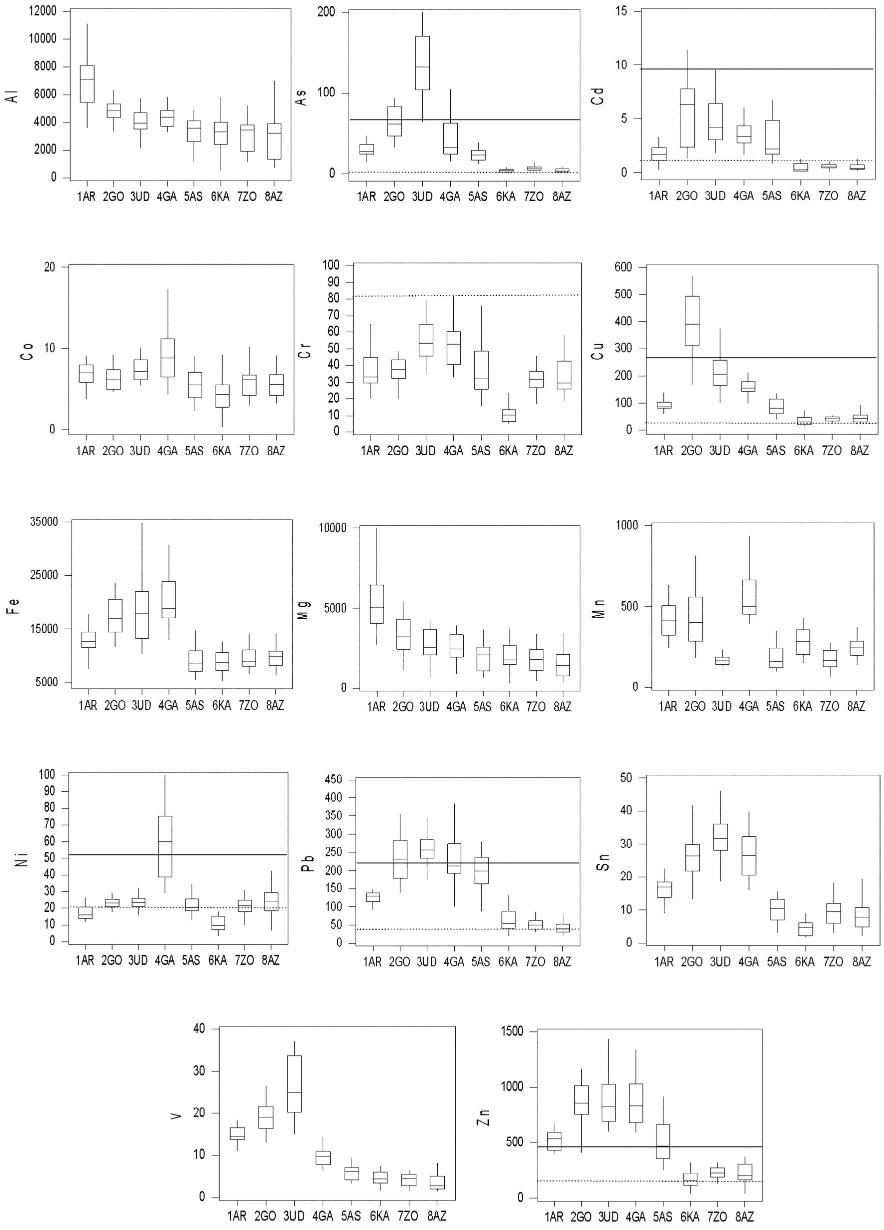

Figure 3. Box-Whisker plots of the trace element concentrations (mg·kg^{-1}) measured in sediments collected between January 2005 and October 2010 at each sampling site. The box shows the 25th percentile and the 75th percentile, and the whiskers represent the lowest and the highest concentrations, while the line inside the box expresses the average value. The discontinuous line indicates the ERL concentration and the continuous one the ERM concentration defined for each metal.

The results shown in Figure 3 and Table 1 were used to investigate similarities among the behaviour of metals, regarding their origin or common pathways controlling their occurrence in sediments.

Table 1. Spearman correlation matrix obtained after the analysis of the whole data set (January 2005-October 2010). Correlations significantly different from zero at a 99% confidence level ($\rho > 0.19$) are marked in bold, and the most significant ones ($\rho > 0.64$) are underlined.

	Al	As	Cd	Co	Cr	Cu	Fe	Mg	Mn	Ni	Pb	Sn	V	Zn
Al	1.00													
As	**0.43**	1.00												
Cd	0.10	**0.74**	1.00											
Co	**0.24**	**0.36**	**0.21**	1.00										
Cr	**0.32**	**0.68**	**0.62**	**0.37**	1.00									
Cu	**0.35**	**0.87**	**0.80**	**0.30**	**0.64**	1.00								
Fe	**0.32**	**0.72**	**0.57**	**0.34**	**0.67**	**0.72**	1.00							
Mg	**0.47**	**0.40**	0.18	**0.38**	**0.23**	**0.34**	**0.35**	1.00						
Mn	**0.30**	0.14	0.14	**0.25**	0.12	**0.25**	**0.48**	**0.31**	1.00					
Ni	0.07	**0.34**	**0.36**	**0.41**	**0.67**	**0.39**	**0.44**	0.16	**0.19**	1.00				
Pb	**0.38**	**0.89**	**0.75**	**0.31**	**0.68**	**0.84**	**0.67**	**0.34**	0.17	**0.39**	1.00			
Sn	**0.48**	**0.86**	**0.64**	**0.40**	**0.78**	**0.86**	**0.77**	**0.38**	**0.23**	**0.49**	**0.81**	1.00		
V	**0.42**	**0.87**	**0.59**	**0.31**	**0.53**	**0.77**	**0.66**	**0.46**	**0.21**	0.13	**0.69**	**0.79**	1.00	
Zn	**0.39**	**0.85**	**0.84**	**0.35**	**0.67**	**0.82**	**0.72**	**0.39**	**0.26**	**0.44**	**0.85**	**0.82**	**0.70**	1.00

Although not very high correlation was found between Al and Mg (ρ=0.45), they presented similar spatial distribution along the estuary (see Figure 3), with a decreasing concentration profile as the distance from the mouth increases. This fact could indicate a common source located in the surroundings of the Abra Bay (where the estuary flows into the ocean) or, simply a direct relation of their concentration with the salinity of water. Fe, Sn, Zn, and Pb presented a similar distribution pattern along the estuary, with high concentrations in *GO*, *UD* and *GA* (also in *AS* for Zn and especially for Pb), low in *KA, ZO* and *AZ* (also in *AS* for Fe and Sn) and intermediate values in *AR*, and they are all together significantly and positively correlated (ρ between 0.67 and 0.85). As, Cd, Cu and V, which were also positively correlated, presented similar spatial distribution with significantly higher concentrations in *GO* and *UD*. The Co and Ni concentrations measured in the sediments did not differ significantly from site to site, except in *GA* where both metals (especially Ni) presented higher concentration values. The spatial distribution of Cr and Mn is completely different to the rest of the metals. Cr concentration is high and quite uniform all over the estuary, with the exception of *KA* where low concentrations were found. This is the only toxic element which shows relatively high concentrations in the most upper sites, e.g., *ZO* and *AZ*. The presence of Mn is relatively higher in *AR*, *GO* and *GA* than in the rest of the sites, and surprisingly low in *UD*. Mn is not highly correlated with the rest of the elements, but surprisingly Cr presents relatively high positive correlations with As (0.68), Cu (0.64), Fe (0.67), Ni (0.67), Pb (0.68), Sn (0.78) and Zn (0.67).

Summarising, the sediments with higher content in toxic elements like As, Cd, Cr, Cu, Ni, Pb and Zn are concentrated in the sites influenced by the Gobela River (*GO, UD*), and *GA* (which is probably affected by the sewage treatment plant located in its banks). Sediments from the upper part of the estuary (*AZ, KA* and *ZO*) are less affected by metal pollution, with the exception of Cr. A remarkable high concentration of Pb is characteristic of the samples from *AS*, with the rest of elements in this site in or below the average of the other sampling points. The samples from *AR* show a differentiated fashion, with sediments especially rich in Al, Mg and Mn.

3.4. Temporal distribution of trace metals

The concentrations found in each sampling campaign are shown in the form of Box-Whisker plots in Figure 4. Generally speaking, only slight decreasing trends were observed with time for Cd and Cr, and increasing ones for Co and Mg. The Mann-Kendall Test was again applied to find possible trends in the time series profiles (from January 2005 to October 2010) of trace elements in each sampling site. For clarity, only trends detected at a confidence level over 95% have been considered in Table 2. Only Co, Ni and Mg concentrations slightly increased (95%) with time in sediments of the most upper stations (those with low metal concentrations), e.g., *KA* (Co), *ZO* (Ni) and *AZ* (Co and Mg). Among these sites only one decreasing trend was observed for As in *ZO* at a confidence level of 99%. For the rest of sites and metals, when a trend was detected it was always negative. This fact suggests that sediments with high metal content may be acting as secondary contamination source in the estuary.

A similar study was performed previously[15] using a sub-set of the data (January 2005 to January 2008). Comparison of the results obtained in that period with those summarised in Table 2 show i) that both the increasing trends in *KA, ZO* and *AZ*, and the decreasing ones in *UD*, *GO* and *AS* are not so marked in the last three years of monitoring than in the previous ones, and ii)

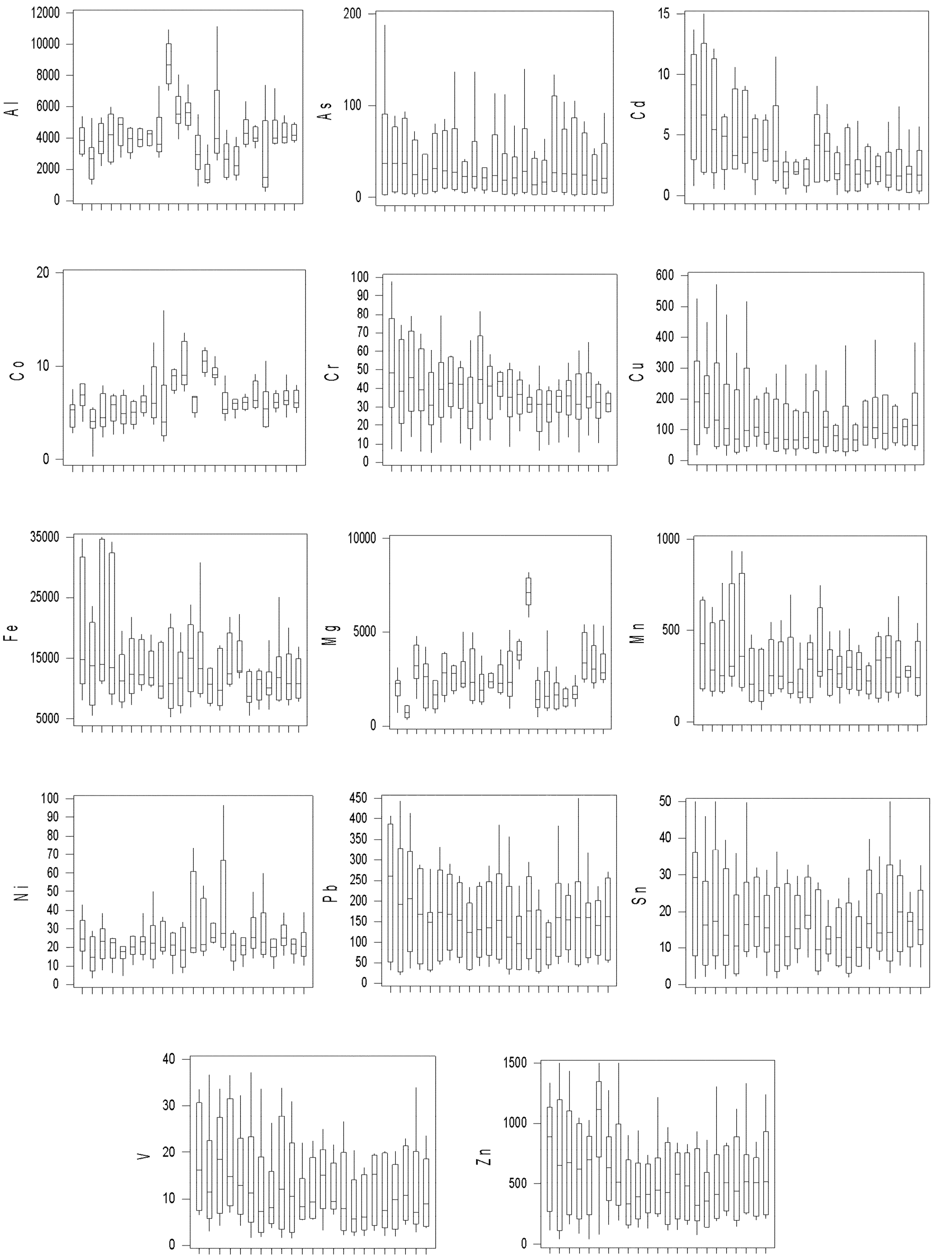

Figure 4. Box-Whisker plots of the trace element concentrations (mg·kg^{-1}) measured in sediments collected between January 2005 and October 2010 in each sampling campaign. The sampling campaigns are ordered from left to right in the X axis (January 2005 to October 2010). The box shows the 25th percentile and the 75th percentile, and the whiskers represent the lowest and the highest concentrations, while the line inside the box expresses the average value.

Table 2. Increasing (+) and decreasing (-) trends detected by the Mann-Kendall test in the time series (Jan 2005-Oct 2010) of each element at each sampling site. 95, 99 and 99.9 express the confidence level at which the trend was detected.

	AZ	***ZO***	***KA***	***AS***	***GA***	***UD***	***GO***	***AR***
Al								
As		(-)99		(-)95		(-)99.9		(-)99.9
Cd				(-)99.9		(-)99	(-)99	
Co	(+)95		(+)95					
Cr				(-)99	(-)99	(-)99.9		
Cu						(-)99		
Fe				(-)95		(-)99.9	(-)95	
Mg	(+)95							
Mn								(-)95
Ni		(+)95		(-)95				
Pb						(-)99.9	(-)95	
Sn						(-)99		
V						(-)99.9		
Zn						(-)95		

that the increasing profiles observed in the *GA* sampling site for Ni and Co from January 2005 to January 2008 have ceased. It should be noted, in addition, that in regard to the sites with decreasing trends from Jan 2005 to Jan 2008 (*UD*, *GO* and *AS*) there are three different patterns of behaviour. In a first case the decreasing trend follows until Oct 2010 (As and Cd in *UD*, for example). A second pattern corresponds to a cease of the decreasing trend with a maintenance in metal concentration over the last three years (Mn in *AS*). Finally, there are several sites in which the concentration of a metal even increases from April 2008 to October 2010 (Sn in *GO*).

3.5. Geoaccumulation indexes

Metal concentrations found in other estuaries of the world are given in Table 3 as a guideline. Comparison with the results obtained here, however, is not easy due to the specific geochemical characteristics of each emplacement and to differences in the methods used for sediment analysis. The geoaccumulation indexes, which compare the current pollutant concentration of the sediment with the pre-industrial or natural background calculated for the zone, allow a preliminary estimation of the anthropogenic fraction in the pollutant content, and provide, consequently, a common scale for inter-estuary comparison.

Table 3. Concentrations (in $\mathrm{mg \cdot kg^{-1}}$) reported for several elements in different estuaries of the world.

Estuary	As	Cd	Cr	Cu	Fe
Salt-Water (Taiwan)		1.4	131	1001	41868
Jobos (Puerto Rico)	16.0-18.0	0.05-0.1	-	26.0-32.0	-
Ennore Creek (India)	-	0.43-1	44.0-86.0	20.0-33.0	1983-2950
Nanliu (China)	18.1	0.1	45.3	68.4	-
Odiel (Spain)	330	14.4	-	2109	-
Tinto (Spain)	339	8.4	-	1897	-
Canal del Santo padre (Spain)	384	7.1	-	2215	-
Cochin (India)	-	7.0	-	1346	44.5
Minho (Portugal)	-	0.02-0.3	-	2.8-22.4	1.4-3.9
Douro (Portugal)	-	0.1-0.3	-	1.0-229	0.27-3.0
Tagus (Portugal)	-	0.9-11.0	-	9.7-214	3.1-5.0
Tamar (England)	-	0.4-0.5	-	129-161	30500-36500
Poxim (Brazil)	-	0.06-0.4	3.0-13.0	5.3-12.3	-
Bohai Bay (China)		0.16-0.18	53.0-63.0	26.0-33.0	-
Gao-ping (Taiwan)	11.2	0.1	57.5	32.8	-
Patras Harbour (Greece)	8.8	0.5	202	82.5	35000
Thermaikos (Greece)	-	-	47.0	87.0	-
Restronguet (England)	-	-	-	2890	-
Drin (Albania)	-	0.10-0.13	-	12.0-13.0	725-749
Mahanadi (India)	-	1.4	3.1	4.0	141
Gironde (France)	18.7	0.50	78.4	24.5	-
Nerbioi-Ibaizabal ***(Basque Country)***	0.6-219	0.05-17.5	5-134	12.6-769	5527-36715

Table 3 (continued). Concentrations (in $\mathrm{mg \cdot kg^{-1}}$) reported for several elements in different estuaries of the world.

Estuary	Mn	Ni	Pb	Zn	Ref.
Salt-Water (Taiwan)	639	103	128	1220	47
Jobos (Puerto Rico)	-	-	10.0-12.0	58.0-70.0	48
Ennore Creek (India)	197-321	14.0-27.0	30.0-45.0	100-135	49
Nanliu (China)	-	-	34.0	57.4	50
Odiel (Spain)	-	-	590	1154	51
Tinto (Spain)	-	-	496	1115	51
Canal del Santo padre (Spain)			630	1431	51
Cochin (India)	4.0	70.6	27.2	578	52
Minho (Portugal)	-	-	4.8-15.9	37.7-91.6	53
Douro (Portugal)	-	-	0.25-192	6.2-457	53
Tagus (Portugal)	-	-	11.8-350	88.2-1086	53
Tamar (England)	348-390	25.0-28.0	139-165	246-282	54
Poxim (Brazil)	-	0.30-3.0	8.1-15.9	7.4-30.6	55
Bohai Bay (China)	-	31.0-36.0	24.9-26.3	72.3-96.7	56
Gao-ping (Taiwan)	-	34.0	24.9	122	57
Patras Harbour (Greece)	1015	110	49.5	120	58
Thermaikos (Greece)	-	-	77.0	184	59
Restronguet (England)	-	-	168	2200	60
Drin (Albania)	289-299	-	0.40-0.50	6.9-7.1	61
Mahanadi (India)	182	8.0	7.9	20	62
Gironde (France)	-	32.0	46.8	168	63
Nerbioi-Ibaizabal ***(Basque Country)***	67-936	3.4-935	21-458	41-2060	This study

The background values defined by Cearreta et al.[24] for As (16 $mg\cdot kg^{-1}$), Cr (85 $mg\cdot kg^{-1}$), Cu (20 $mg\cdot kg^{-1}$), Fe (25000 $mg\cdot kg^{-1}$), Mn (300 $mg\cdot kg^{-1}$), Ni (23 $mg\cdot kg^{-1}$), Pb (21 $mg\cdot kg^{-1}$) and Zn (63 $mg\cdot kg^{-1}$), and Rodríguez et al. [29] for Cd (0.24 $mg\cdot kg^{-1}$), were used to calculate the corresponding geoaccumulation indexes by the formula proposed by Müller[9].

According to the scale established by this author an area can be classified as unpolluted ($I_{geo}<1$), very slightly polluted ($1 < I_{geo}<2$), slightly polluted ($2 < I_{geo}<3$), moderately polluted ($3 < I_{geo}<4$), highly polluted ($4 < I_{geo}<5$), and very highly polluted ($I_{geo} > 5$). I_{geo} indexes for each trace element are summarised in Figure 5 in the form of Box-Whisker plots.

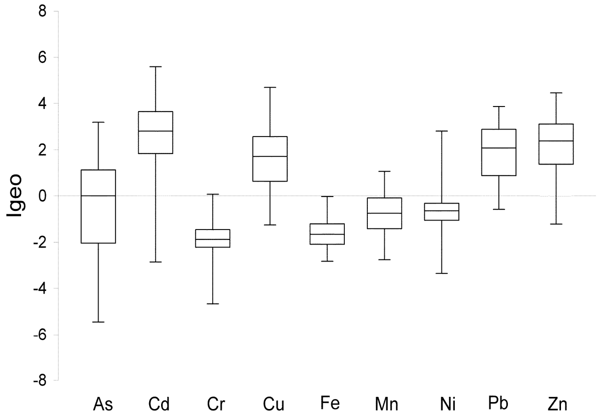

Figure 5. Box-Whisker plots of the geoaccumulation indexes calculated for As, Cd, Cr, Fe, Mn, Ni, Pb and Zn in the sediments of the *Nerbioi-Ibaizabal* estuary. The box shows the 25th percentile and the 75th percentile, and the whiskers represent the lowest and the highest concentrations, while the line inside the box expresses the average value.

Cd presents the highest indexes, with several samples from *GO*, *UD* and *AR* in the very highly polluted level. Several geoaccumulation indexes calculated for Cu and Zn are also close to 5 in several sediments from *AS, GO* and *UD*. Moderate contamination by Pb was very frequent in *GO*, *UD*, *AS* and *GA*, especially at the beginning of the time series. Contamination by As was slight in *GO, UD* and *GA*, and systematically non detectable ($I_{geo}<1$) in *AR*, *AS*, *KA*, *ZO* and *AZ*. Finally, the indexes calculated indicate that sediments are not polluted with Cr, Fe, Mn and Ni. This last observation may result from an overestimation of the background values calculated for these metals or from a poor recovery during the extraction step in the analysis of sediments. Our analytical method assures high extractions (>70%) of Cr, Mn and Ni from sediments, with a Fe recovery of only about 50%.[39] In addition, the ERL values defined for Ni and Cr are below the background concentrations used in this study, and the background value defined for Cr by Rodríguez et al.[29] is significantly lower than that one given by Cearreta et al.[24] All these facts indicate that overestimation may explain the low I_{geo} values found for Ni and Cr, while poor recovery could be responsible in the case of Fe.

3.6. Sediment Quality Guidelines: toxicity assessment

Sediment Quality Guidelines were used as a preliminary approach to account for toxicity in the sediments. SQGs have been widely applied in risk assessment of different materials of environmental concern. They are useful to study the adverse effects that contaminants could cause in living organism as benthic communities. The so-called effects range-low (ERL) and effects range median (ERM) are probably the most popular SQGs when dealing with sediments.[13] ERL indicates the pollutant concentration below which effects are expected to be rare, and ERM the concentrations above which effects are expected to be frequent. Details on how these criteria have been developed can be consulted elsewhere.[64] ERL and ERM values (in $mg \cdot Kg^{-1}$) have been only defined for very toxic elements such as As (8.2, 70), Cd (1.2, 9.6), Cr (81, 370), Cu (34, 270), Ni (20.9, 51.6), Pb (46.7, 218) and Zn (150, 410). These threshold values have been plotted in Figure 3 together with the basic statistics of the concentrations found in each sampling station. For As and Cd all the sediments, except those from *KA*, *ZO* and *AZ*, are over the ERL value, but only the sediments from *UD* and a few from *GO* exceed the ERM for As. The concentrations found for Cr are all below the ERL defined for this metal. For Ni, only the sediments from *GA* are above the estimated ERM, and the rest of sediments are close or even below the ERL value. Cu, Zn and Pb are generally all over the ERL concentration. In addition, concentrations over the ERM were found for Pb in *GO*, *UD, GA* and *AS*, for Zn in *AR*, *GO*, *UD, GA* and *AS*, and for Cu in *GO*.

The SQGs only consider the individual effect of each contaminant, so that they do not take into account the possible synergic effect of a mixture of pollutants simultaneously present in the sediment. The use of mean sediment quality guidelines quotients (mSQGq) has been proposed to solve this problem. Thus, the mERMq for each sampling site in each sampling campaign were calculated in this work, by dividing each trace element concentration by its respective ERM and averaging the resulting ratios.[13] An easily interpretable single quotient, directly related to the probability of a sample to be toxic, was consequently obtained for each station and campaign. So, mERMq values in the range of 0-0.1, 0.1-0.5, 0.5-1.5, and >1.5 indicate a probability of being toxic of, 9% (non-toxic), 21% (slightly toxic), 49% (moderately toxic) and 76% (highly toxic), respectively.

The evolution in time of the mERMq's calculated for each sampling site is shown in Figure 6. Different trends can be distinguished. A dramatic decrease in toxicity with time can be observed in the case of sediments collected in *UD*, *GO* and especially *AS*. From mERMq's close (*GO*) or even over 1.5 (*AS*, *UD*) in 2005 we go to values between 0.5 and 1 (*GO*, *UD*), and even below 0.5 (*AS*) in 2010.

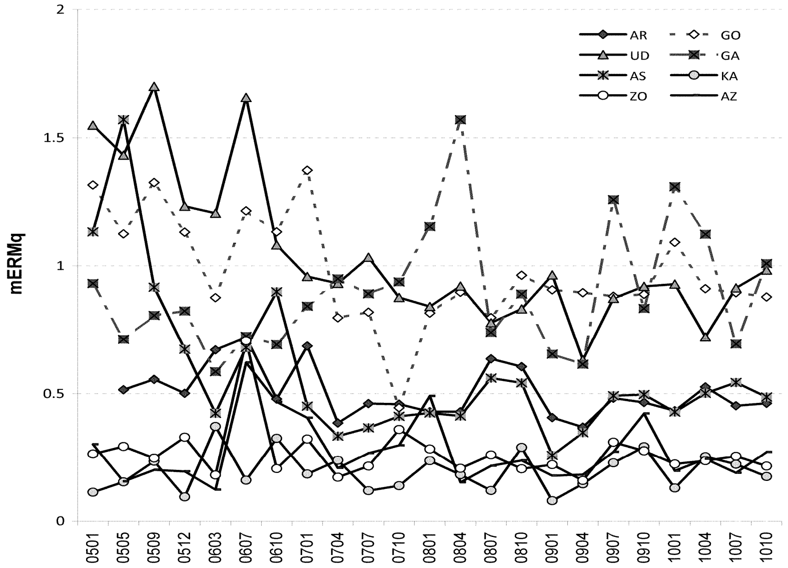

Figure 6. Evolution in time of the mERMq values calculated for the sediments of each sampling site (from January 2005, 0501, to October 2010, 1010).

On the other hand, after an initial decrease until October 2006, the toxicity of the sediments from *GA* seems to increase in time, with a maximum in April 2008 probably due to the high concentration of Ni found in the sediment in that campaign. Finally, while toxicity of sediments from *AR* slightly decrease with time (from moderately toxic to non-toxic), that of *KA*, *ZO* and *AZ* remains more or less constant in relatively low values (~0.25). Very broadly speaking, a relative seasonal trend can be appreciated in the sediments from these sites, with maximum values of mERM quotients in dry season.

4. Conclusions

The geochemical characteristics of the sediments in the estuary do not significantly differ from site to site nor within sampling campaigns as it was confirmed by PCA (Principal Component Analysis) of the data set after excluding the metal concentrations found in each sample. Only conductivity is systematically higher as we move towards the ocean, a fact clearly related to the salinity of water. Pollution due to very toxic elements, such as As, Cd, Cu, Ni, Pb, Sn, V and Zn, however, concentrates nowadays in the area of influence of the Gobela tributary river (*GO* and *UD*), and in the surroundings of the Waste Water Treatment Plant (WWTP) of Galindo (*GA*). Chromium is an exception to this general rule, with a quite homogenous concentration all over the estuary, but still far from the values reported to be potentially toxic. The presence of these elements in the sediments of the estuary, consequently, seems to be mainly due to anthropogenic activity and more related to point sources of pollution rather than to diffuse ones. Diffuse pollution, however, should not be underestimated considering the relatively high amount of trace elements released by local enterprises to the atmosphere, as declared to the European Pollutant Release and Transfer Register (E-PRTR, http://prtr.ec.europa.eu/Home.aspx). The areas mentioned above (*GO*, *UD* and *GA*) presented, especially in the oldest sampling campaigns, sediments with concentrations of As, Cd, Cu, Ni, Pb and Zn well over the natural backgrounds estimated for the area, that in most of the cases (except Cd) may represent, according to the SQGs used in this work, a real threat to living organism from a toxicological point of view.

Broadly spoken, the metal content in sediments of the estuary has decreased within the period investigated so, regarding metal pollution, the actuations implemented in the framework of the so-called “Strategy for the integral recovery of the estuary of Bilbao” seem to have been successful. However, the site of *GA* constitutes an exception to this general rule. The As, Cd and Zn concentrations in sediments from this site slightly but continuously increase with time. An increasing trend is also detectable for Ni and Co at this site until the end of 2007, but from 2008 a clear decrease in the concentration is observed. The WWTP of Galindo, located 500 m upstream our sampling point, treats about 350000 m^3 of waste water per day, including sewage water from most of the industrial facilities located in the surroundings of the estuary and, despite a correct function of the plant, it may discharge an important mass of metals to the estuary. For example, the WWTP declared to the E-PRTR a release to the river of about 1500 Kg of Ni (with a Threshold for Release to

Water, TRW, of 20 Kg per year), 32 Kg of Cd (TRW of 5 Kg per year), 280 Kg of Cr (TRW of 50 Kg per year), 1700 Kg of Cu (TRW of 50 Kg per year) and 4100 Kg of Zn (TRW of 100 Kg per year) in 2009. Thus, the operation of the WWTP should be carefully controlled in the future in the framework of a sustainable management of the estuary.

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Metal pollution in estuarine sediments: from the local scale to a wide world perspective. A case study: the estuary of the Nerbioi-Ibaizabal River (Bilbao, Basque Country)

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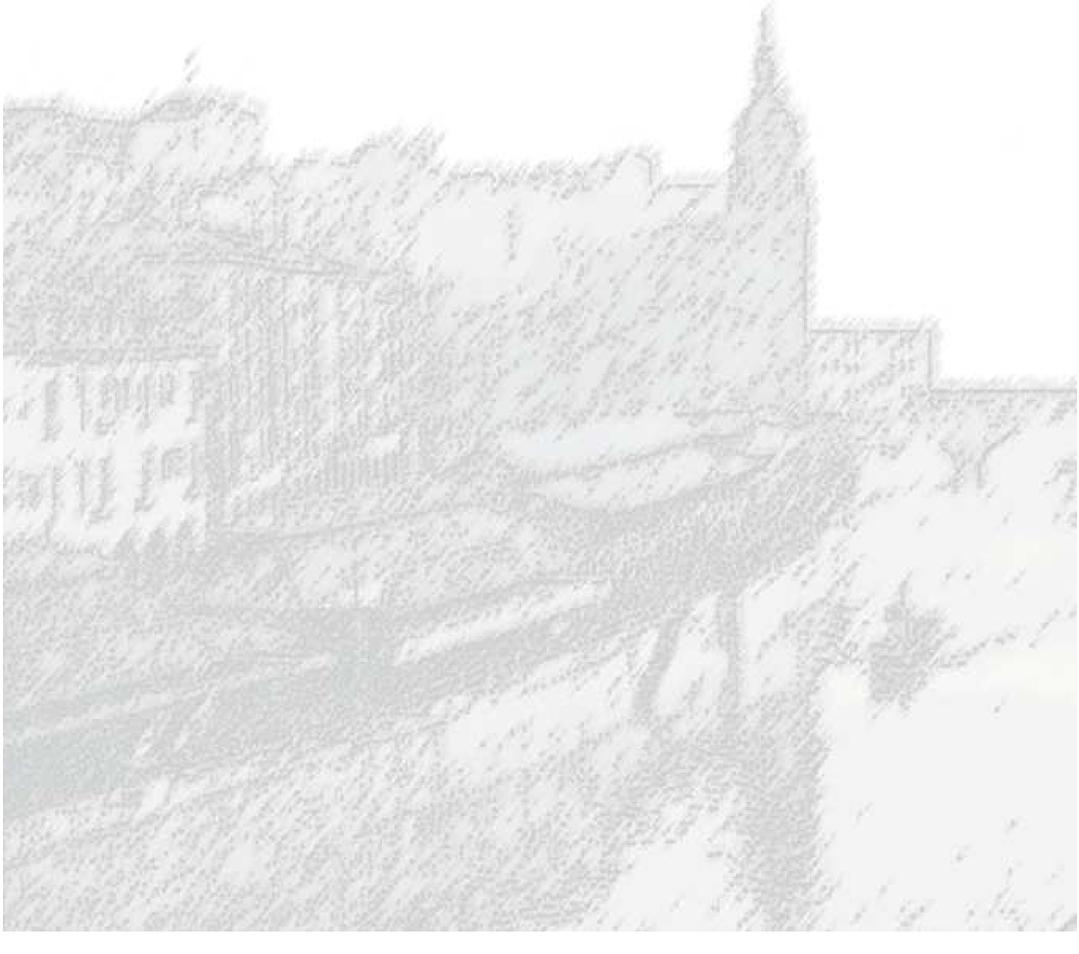


Metal pollution in estuarine sediments: from the local scale to a wide world perspective. A case study: the estuary of the Nerbioi-Ibaizabal River (Bilbao, Basque Country)

Abstract

The search for a simple way to assess pollution in sediments has been a constant challenge during the last decades. Initially, results from chemical analysis were only taken into account. Nowadays, it is generally accepted that both chemical and toxicological data is required to draw realistic conclusions. In this work we propose the combination of different approaches to provide decision makers with a very simple and intuitive tool to classify estuary sediments according to their pollutant content and/or toxicity. The methodology has been exemplified in a case study, e.g., the metal pollution in the estuary of the *Nerbioi-Ibaizabal* River (Bilbao, Basque Country). Sediments were collected at 49 points of the estuary in two sampling campaigns (January 2009 and January 2010). The concentration of 14 elements was measured and the Normalized Average Weighted Concentrations (NAWC), geoaccumulation indexes (I_{geo}) and mean Effect Range-Median quotients (mERMq) were calculated. The results obtained are complementary and allow intra- and inter-estuary comparison. Critical discussion on the strengths and limitations of each indicator is provided, and the potentiality of the proposed methodology to be applied in risk assessment at the local scale is stressed.

Keywords: Sediment; Metal pollution; Geoaccumulation index; Sediment Quality Guidelines; Risk assessment.

1. Introduction

Estuaries are considered the most productive marine ecosystems. These transition areas present high capacity to feed and bring up many organisms, but unfortunately they also store different kinds of pollutants, especially in their sediments [1]. Sediments are complex matrices able to accumulate over time many organic and inorganic substances. Among these chemicals trace elements (metals and metalloids) are of special interest due to their high toxicity and long persistence in the environment. In non-polluted sediments, trace elements present low natural concentrations and quite stable forms (mainly silicates). The presence of metals in sediments, however, significantly increases due to human activities like industrial, mining and waste water works [2-4]. In polluted areas metals are usually associated to mineral phases with higher mobility (aluminium silicates, Fe/Mn hydroxides and carbonates), organic matter and/or suspended particles [5, 6]. However, although the main part of the metal present in the sediments is not available to organisms [7, 8], punctual, seasonal or long-term variations in the physico-chemical properties of the water column (pH, salinity, redox potential, dissolved oxygen or concentration of organic chelators) may reverse this situation, becoming sediments a potential secondary source of metals to the estuary [9-11]. Another problem related to the presence of trace elements in sediments is the biomagnification process that may happen along the food chain under appropriate conditions, which may result in formation of free radicals in microorganisms [12] and DNA damage [13, 14], among others.

Keeping in mind that they suffer small spatial and temporal variation (which allows an effective control of contamination patterns), sediments have been commonly used as indicators of environmental quality in aquatic ecosystems [10, 11, 15, 16]. Chemical analysis has been for a long time the only tool used to investigate the quality of sediments, and hence of the aquatic environment involved. This classical approach does not take into account the effect of possible interactions among pollutants and neither their effect in living organisms [17]. For this reason, the development of tools to link the concentration of different trace elements and their ability to behave like toxic is considered essential nowadays [18]. In fact, the importance of discerning between contamination (pollutant concentration above the background value) and pollution (pollutant concentration which represent a toxicological risk) has been stressed [19]. In addition, as natural composition (background concentrations) largely changes from area to area, the simple pollutant concentration does not provide us with an effective way to compare contamination rates of different environments.

In this context, a wide range of tools has been proposed to evaluate the quality of sediments. The geoaccumulation index (I_{geo}) was defined by Mueller (1981) and has been successfully used as a criterion to assess metal contamination of sediments in many estuaries around the world [20-22]. It provides a valuable tool to compare the contamination rate of different locations taking into account the local natural concentrations as a reference. Other similar approaches have also been proposed [23-25]. The I_{geo}, however, is not able to check possible adverse biological effects due to the pollutant content of sediments. The use of Sediment Quality Guidelines (SQGs) has been proposed to correct this. The SQGs are accepted as important tools for the protection and management of marine ecosystems [26, 27]. Several SQGs have been derived with a variety of empirical methods that rely upon the biological effects data [28]. The most popular ones include the effects-range median (ERM) [29], the probable effects levels (PEL) [30] and probable effects concentrations (PEC) [31]. With the aim to provide robust and simple tools for the management of contaminated sediments, the use of mean sediment quality guideline quotients (mSQGq) has also been proposed [28, 32, 33].

In this work we propose a journey from the local scale to a wide world perspective concerning metal and metalloid pollution in estuarine sediments. The main objective of the study is to provide authorities and decision makers with an easy tool to measure the real magnitude of the concrete problem they have in hands; a tool that combines information from crude chemical analysis with toxicological risk, and that takes into account the specific characteristics of each particular emplacement and the simultaneous effect of multiple pollutants. A case study, the estuary of the *Nerbioi-Ibaizabal* River (Bilbao, Basque Country), has been used to exemplify the methodology proposed. This paper also explores the possibility to obtain further information on the spatial distribution of several elements in the sediments of this estuary.

2. Materials and methods

2.1. Study area

Bilbao is nowadays one of the most important urban areas in the Cantabrian coast with about one million inhabitants. Exploitation of local iron, starting at the end of XIXth century, marked the beginning of an important industrial activity, mainly centred in the manufacture of steel, electroplating and ship construction, which resulted in a dramatic increase in population. Until the middle seventies all the wastewater from urban and industrial origin was directly dumped into the estuary, which provoked the environmental collapse of the system [11, 34, 35]. However, the situation of the estuary improved significantly at the end of the XXth century due to the closure of the most polluting industries and the design and implementation of the so-called “strategy for the integral recovery of the estuary of Bilbao”. The actuations started in 1979 and have almost finished with spectacular results [36, 37]. The sediments of the estuary, however, faithfully reflect historical pollution episodes, and their pollutant content, specifically in trace metals and metalloids, has been reported to be important [38-45]. In such situations, the European Water Framework Directive recommends the implementation of a surveillance monitoring programme to follow the evolution of pollution in time and space.

49 sampling sites were strategically selected along the estuary in order to obtain an appropriate representation of the studied area (Figure 1). Sediments were collected in the main flow of the estuary (including semi-closed docks), tributary rivers, upstream and downstream the effluent-output of the most important sewage plant of the area, and also in the mouth of the estuary (Abra Bay).

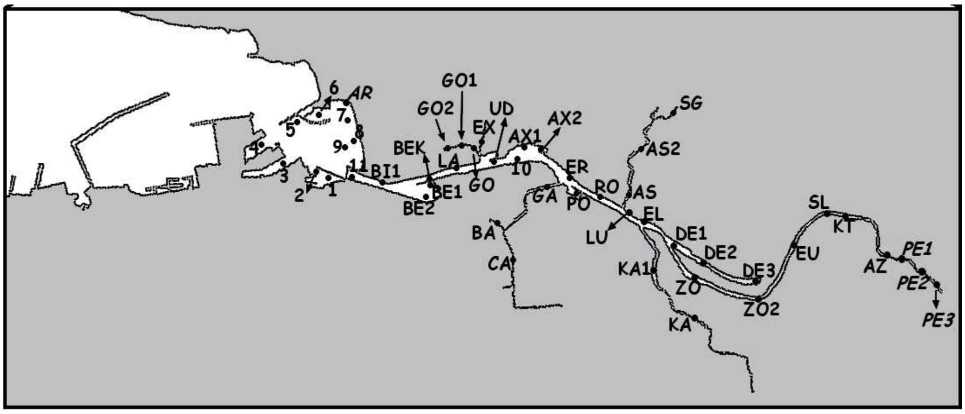


Figure 1. Sampling sites in the estuary of the *Nerbioi-Ibaizabal* River.

2.2. Methods

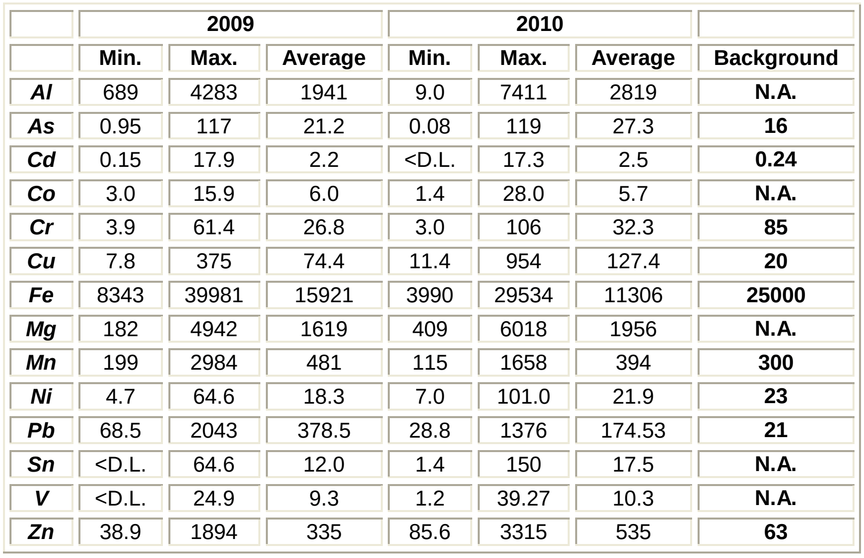
Two sampling campaigns were carried out in January 2009 and January 2010. In most of the cases, surface sediments (0-2 cm) were collected at low tide from the banks by hand using plastic gloves. Exceptionally, sediments at the sampling points 1-11 were collected from a boat using a Van Veen type grab. Samples were transported to the laboratory inside plastic bags at 4 ºC to reduce the microbiological activity. Before analysis, the sediments were frozen at -20 ºC and lyophilized at 150 mTorr and -52 ºC in Cryodos apparatus (Telslar, Spain) for 48 h. The dry samples were sieved and only the fraction <63 μm was retained. The concentration of Al, As, Cd, Co, Cr, Cu, Fe, Mg, Mn, Ni, Pb, Sn, V and Zn was measured in the fine fraction by ICP/MS (Perkin-Elmer, Elan 5000) after the focused ultrasound assisted extraction of the sediment with a HNO_3/HCl mixture as extractant. More details about the analytical procedure can be found elsewhere [46].

3. Results and discussion

3.1. Classification of sampling sites according to raw metal concentrations

The average, maximum and minimum concentrations of the elements measured (Al, As, Cd, Co, Cr, Cu, Fe, Mg, Mn, Ni, Pb, Sn, V and Zn) in the sediments of the estuary of the *Nerbioi-Ibaizabal* River are summarised in Table 1.

Table 1. Maximum, minimum and the average concentrations (mg·kg^{-1}) found within sampling sites in sediments of the estuary of the *Nerbioi-Ibaizabal* River. Background values (mg·kg^{-1}) reported for the area and detection limits (mg·kg^{-1}) in the analysis of each element are also provided (<D.L.: below detection limt ; N.A.: information not available).



	2009			**2010**			
	Min.	**Max.**	**Average**	**Min.**	**Max.**	**Average**	**Background**
Al	689	4283	1941	9.0	7411	2819	**N.A.**
As	0.95	117	21.2	0.08	119	27.3	**16**
Cd	0.15	17.9	2.2	<D.L.	17.3	2.5	**0.24**
Co	3.0	15.9	6.0	1.4	28.0	5.7	**N.A.**
Cr	3.9	61.4	26.8	3.0	106	32.3	**85**
Cu	7.8	375	74.4	11.4	954	127.4	**20**
Fe	8343	39981	15921	3990	29534	11306	**25000**
Mg	182	4942	1619	409	6018	1956	**N.A.**
Mn	199	2984	481	115	1658	394	**300**
Ni	4.7	64.6	18.3	7.0	101.0	21.9	**23**
Pb	68.5	2043	378.5	28.8	1376	174.53	**21**
Sn	<D.L.	64.6	12.0	1.4	150	17.5	**N.A.**
V	<D.L.	24.9	9.3	1.2	39.27	10.3	**N.A.**
Zn	38.9	1894	335	85.6	3315	535	**63**

The results obtained are in good agreement with previous measurements [40, 47-49]. The spatial distribution in the estuary considerably changes from element to element. As an example, the distribution profiles obtained for Al, Cu and Fe are shown in Figure 2.

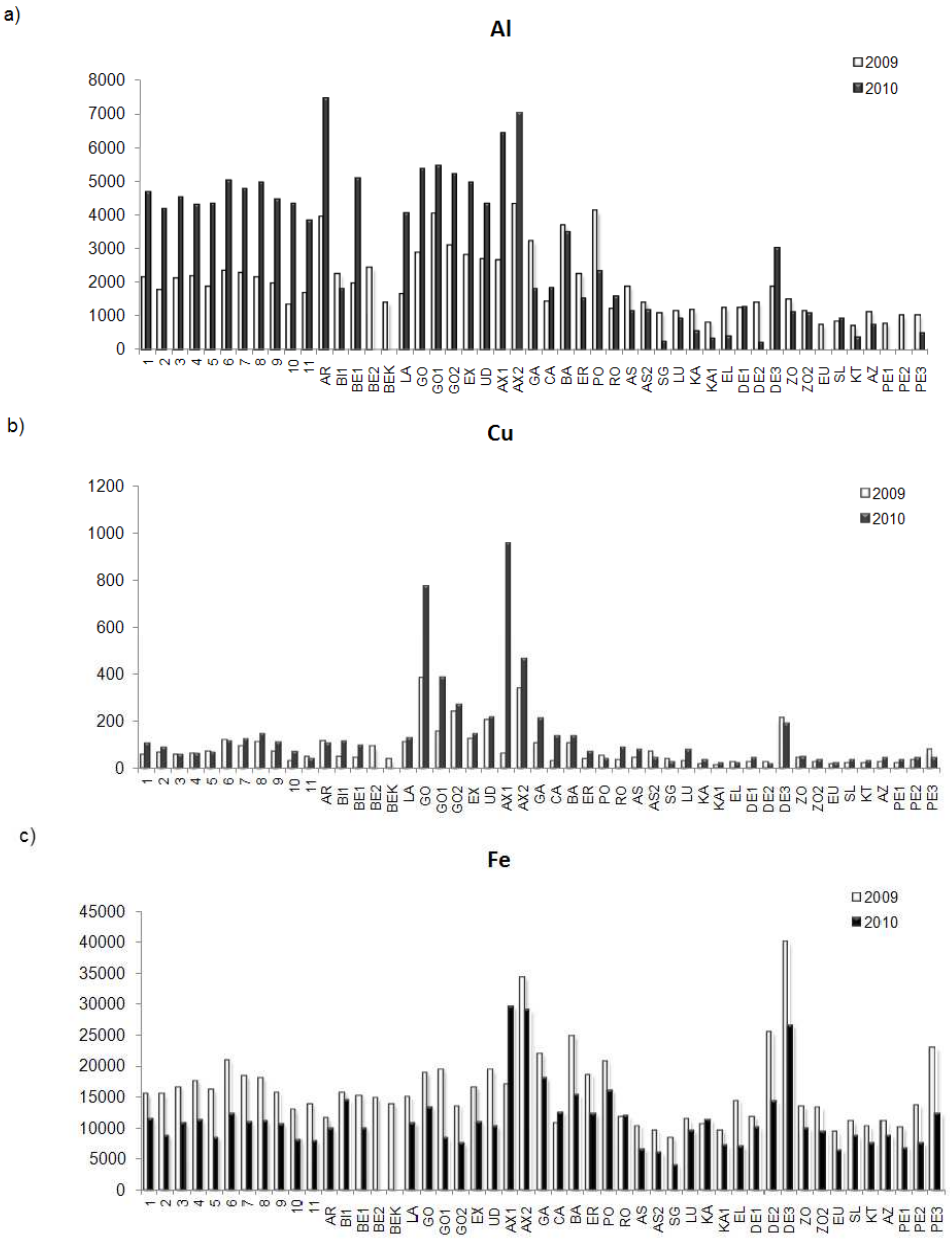


Figure 2. Concentration (mg·kg^{-1}) profiles of Al, Cu and Fe along the estuary in January 2009 and January 2010.

Iron presents relatively homogeneous distribution along the estuary. Contamination by Cu is mainly focused in the Gobela tributary and the Axpe semi-closed dock. Al, however, shows a decreasing trend as we move from the Abra Bay to the upper part of the estuary.

In a first approach, the raw concentrations were used to obtain a simple and intuitive classification of the sampling sites according to the average metal content of their sediments. The goal was to obtain a single score, in a zero-to-ten scale, for each sampling site. First, the concentrations found for each element within sampling sites were normalized by linear scaling transformation ($c_{norm} = (c-c_{min})/(c_{max}-c_{min})$). Then, a weight was calculated for each element according to the distribution of the concentrations of that element within sampling sites. When a wide distribution was found – which means a high capacity to discern among sampling sites – a high value of weight was adopted. In fact, the standard relative deviations (RSD) calculated for each element were normalized (RSD_{norm}) by linear scaling transformation ($RSD_{norm} = (RSD-RSD_{min})/(RSD_{max}-RSD_{min})$) and the resulting numbers from 0 to 1 were used to weight the normalized concentrations (c_{norm}) of the elements. The obtained normalized weighted concentrations (c^{w}_{norm}) were averaged for each sampling site ($\overline{c^{w}_{norm}}$). The averaged values were again normalized from 0 to 1, and finally multiplied by 10 to obtain a score (the Normalized Average Weighted Concentration, NAWC) for each sampling site ranging from 0 to 10.

The results obtained for each sampling campaign (2009 and 2010) are shown in Figure 3. It is to be highlighted that the sites located in the most upper part of the estuary (upstream the *ER* sampling point) systematically presented the lowest score values. This includes sites located in the main channel, in tributary Rivers (Asua and Kadagua) and docks (Portu, *PO*). The most inner part of the close channel of Deustu, *DE3*, constitutes the exception, with high score values in 2009 and 2010.

The most conflictive sites are in the middle estuary, from *ER* to *LA* sampling points. The sediments collected in the Gobela (*GO*, *GO1* and *GO2*) and Galindo (*GA* and *BA*) Rivers, the Axpe (*AX1* and *AX2*) and Udondo (*UD*) semi-closed docks and the Lamiako sampling point (*LA*) showed the highest scores both in 2009 and 2010. The case of the Galindo River should be considered in more detail.

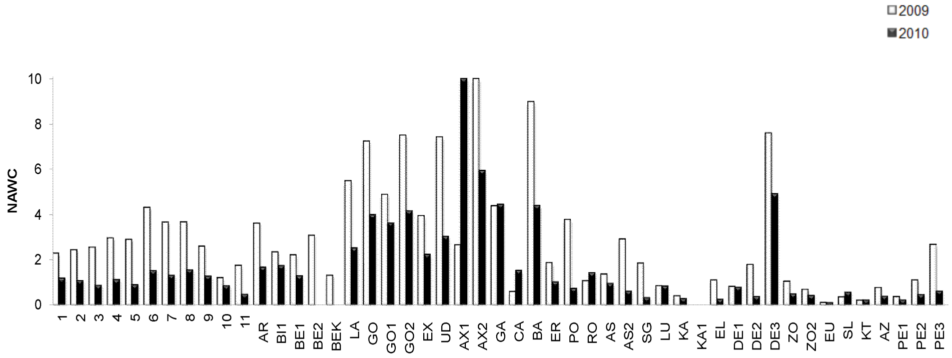


Figure 3. Normalized Average Weighted Concentration (NAWC) values calculated for each sampling site of the estuary in January 2009 and January 2010.

The Castaños and Ballonti tributaries join to form the Galindo River about 200 m upstream a huge waste water treatment plant (WWTP) located in the banks of the river. The output stream of the WWTP flows into the Ballonti tributary. Very high scores were obtained in the *BA* and *GA* sampling sites, and low values however in *CA*. The impact of the WWTP thus becomes evident.

The sites located in the mouth of the estuary (downstream the Benedicta semi-closed dock) presented quite homogeneous intermediate score values. It should be noted, moreover, that the score values obtained for the 2010 campaign are more homogeneously distributed along the estuary than those obtained for the 2009 campaign. This indicates that, in 2010, pollution tended to concentrate in a few points (mainly in *AX1*), rather than to distribute in more points of the estuary (*AX2*, *BA*, *DE3*, *UD*, *GO*, *GO2* and *LA*), like in 2009.

The score values calculated according to the methodology described above are only valid to investigate the spatial distribution of pollution in a selected environment at a given moment. They are site- and time-specific. For example, in the case investigated here, they give us information on the distribution of pollution within sampling points in January 2009 and in January 2010. We can compare the distribution patterns obtained in each campaign, but comparison of the scores obtained in each campaign does not allow us concluding that the magnitude of contamination is higher or lower in one campaign than in the other. They can be used to decide, for instance, where to start with a decontamination process, but they can not be used to monitor the evolution of contamination in time. In addition, the NAWCs do not tell us if sediments are contaminated or not and, as they use no common reference, they do not allow comparison with other estuaries of the world.

3.2. Classification of sampling sites according to the geoaccumulation indexes

In a further step and in an attempt to provide us with a tool to compare pollution in environments of different geochemical characteristics, we calculated the geoaccumulation indexes, I_{geo}, according to Eq. 1 [50]:

$$I_{geo} = \log_2 \frac{c}{1.5\, c_{bg}} \qquad \text{(Eq. 1)}$$

where c is the concentration of a given pollutant and c_{bg} is the background value estimated for that pollutant in the studied area. According to the scale established by Müller, a sediment can be classified as non-polluted ($I_{geo} < 1$), very slightly polluted ($1 < I_{geo} < 2$), slightly polluted ($2 < I_{geo} < 3$), moderately polluted ($3 < I_{geo} < 4$), highly polluted ($4 < I_{geo} < 5$), and very highly polluted ($I_{geo} > 5$).

The geoaccumulation indexes for each element at each sampling site were calculated using background values previously published [47, 51], see Table 1 [47]. Only were reported the background values for eight (As, Cr, Cu, Fe, Mn, Ni, Pb and Zn) of the fourteen elements considered here. The background concentration for Cd was taken from [51]. The obtained I_{geo} indexes are shown in Table 2.

The I_{geo} values found for the 2009 campaign indicate that regarding arsenic most of the sampling sites are classified as non-polluted or very slightly polluted. Only *LA, UD, DE3* present values in the slightly polluted range. In 2010, the situation is similar, but a few more sites (*LA*, *UD*, *AX1*, *AX2*, *GA*) are classified as slightly polluted.

Pollution by cadmium is important in the estuary and it is mainly focused in the middle part of the estuary and in the Abra Bay. Particularly high values of I_{geo} have been found in *GO1, GO2, UD, AX2, BA, GA, AR (*only in 2009*)* and *DE3*. Values above 2 are frequent in this area, especially during the 2009 campaign.

Pollution by copper in the estuary is clearly localized in the surroundings of the Gobela and Galindo tributaries, and the Axpe and Deustu semi-closed docks. In these areas most of the sediments ranged from slightly to moderately polluted with Cu, but highly polluted sediments ($4 < I_{geo} < 5$) were found in the *GO* and *AX1* sampling points in 2010. In general, slightly higher values of I_{geo} were found in 2010 than in 2009.

The results obtained for chromium, iron and nickel should be handled with care. Most of the calculated I_{geo} values are below 0, with only a few ones between 0 and 1. The poor recovery of these elements in the extraction process during the analysis of the sediments or, alternatively, an overestimation of the background values previously calculated for the area are probably behind these unexpected results [46]. In the case of lead, the situation changes significantly from 2009 to 2010. In 2009, the presence of this metal well above the background value reported for the area is a constant all over the estuary. The I_{geo} values calculated for most of the sites located downstream the mouth of the Asua tributary River are over 3, indicating from moderate to very high pollution. Upstream that point, relatively high values (between 1 and 3) were also obtained. The most inner part of the Deustu channel (*DE3*) is also very highly polluted with Pb. In 2010, however, only the sediments from *AX1* were catalogued as very highly polluted, those from *DE3* as highly polluted, and four more as moderately polluted. The rest of sediments presented I_{geo} values below 2.

The spatial distribution of zinc in the estuary is similar to that of Cd but, in general, with lower values of I_{geo}. Most of the sites located upstream the Galindo River (except *DE3*) are not polluted or only very slightly polluted. The problem concentrates, like in the case of Cd, in the Gobela and Galindo tributaries, and the Udondo and Axpe docks, with most of the sediments classified from moderately to highly polluted. In general terms, the situation is similar in 2009 and 2010, with slightly higher values of I_{geo} in the second year.

According to the results obtained, pollution by manganese is not important in the estuary. Only a few sites (*GO2*, *BA*, *PO* and *DE3*) showed values of I_{geo} between 1 and 2 (very slight pollution).

In general terms, and based on the average I_{geo} values ($\overline{I_{geo}}$) calculated for each element in 2009, it can be concluded that at that time the estuary of the *Nerbioi-Ibaizabal* River was moderately polluted with Pb, very slightly polluted with Cd and Zn, and non-polluted with As, Fe, Cr, Cu, Mn and Ni. In 2010, very slight pollution was measured for Cd, Pb and Zn, while the estuary remained as non-polluted for the rest of elements considered.

As it has been above mentioned the geoaccumulation indexes allow us comparing situations in different environments taking into account their specific geochemical characteristics. As an example, the average values of the I_{geo} values calculated for each metal in the estuary of the *Nerbioi-Ibaizabal* River (in 2010) are compared with values estimated for other three aquatic systems of the world in Table 3.

Table 2. Geoaccumultaion index (I_{geo}) values calculated for each sampling site of the estuary in January 2009 and January 2010 (<D.L.: the concentration of the element at that sampling site is below the detection limit; N.A.: the concentration of the element at that sampling site is not available).

	I_{geo}	1	2	3	4	5	6	7	8	9	10	11	AR	BI1	BE1	BE2	BEK	LA	GO	GO1	GO2	EX	UD	AX1	AX2
2010	**As**	-0.45	-0.54	-0.64	-0.19	-0.60	0.51	-0.12	0.33	0.14	-0.88	-1.28	0.20	0.43	-0.39	N.A.	N.A.	2.31	1.15	1.14	0.88	1.36	2.02	1.98	2.20
2009	**As**	-0.84	-0.52	-0.55	-0.40	0.29	0.58	0.27	0.28	-0.44	-2.21	-1.02	-0.73	-1.20	-0.99	-0.39	-1.47	2.25	1.09	-0.69	0.33	1.06	2.27	-0.68	1.15
2010	**Cd**	-2.93	<D.L.	2.10	<D.L.	<D.L.	<D.L.	1.71	1.69	0.61	<D.L.	2.00	-0.11	2.94	0.64	N.A.	N.A.	2.09	2.64	4.95	5.59	<D.L.	3.42	3.32	3.88
2009	**Cd**	2.01	2.01	2.02	1.85	2.15	2.28	2.30	3.29	2.04	0.68	1.84	4.04	2.86	2.69	2.98	1.42	3.49	3.93	3.92	5.63	2.02	3.96	1.79	3.27
2010	**Cr**	-1.86	-2.21	-2.44	-2.35	-2.32	-1.85	-1.94	-1.88	-1.92	-2.36	-2.54	-2.11	-1.77	-1.74	N.A.	N.A.	-1.46	-1.93	-1.68	-2.25	-2.00	-1.27	-0.25	-0.95
2009	**Cr**	-2.11	-2.21	-2.12	-1.95	-2.15	-1.67	-1.77	-1.92	-2.08	-2.73	-2.43	-2.65	-2.30	-2.25	-1.87	-2.44	-1.71	-1.99	-2.39	-2.39	-2.03	-1.28	-2.18	-1.17
2010	**Cu**	1.76	1.47	0.74	0.95	1.01	1.88	2.00	2.22	1.80	1.17	0.15	1.74	1.87	1.59	N.A.	N.A.	2.05	4.68	3.67	3.14	2.23	2.83	4.99	3.94
2009	**Cu**	0.82	0.99	0.83	0.87	1.11	1.90	1.56	1.81	1.11	-0.19	0.51	1.86	0.51	0.34	1.57	0.24	1.78	3.65	2.30	2.97	1.97	2.72	0.87	3.47
2010	**Fe**	-1.70	-2.10	-1.80	-1.74	-2.15	-1.60	-1.78	-1.76	-1.83	-2.22	-2.25	-1.93	-1.38	-1.92	N.A.	N.A.	-1.79	-1.49	-2.15	-2.32	-1.78	-1.86	-0.34	-0.37
2009	**Fe**	-1.28	-1.28	-1.18	-1.09	-1.22	-0.85	-1.03	-1.06	-1.26	-1.54	-1.44	-1.70	-1.25	-1.31	-1.34	-1.44	-1.32	-1.00	-0.95	-1.48	-1.18	-0.96	-1.14	-0.13
2010	**Mn**	-1.21	-1.17	-1.00	-0.56	-1.12	-0.89	-1.11	-0.98	-1.03	-1.24	-1.74	0.10	-0.64	-0.33	N.A	N.A	-1.36	-0.06	0.51	1.84	-0.14	-1.55	0.93	0.27
2009	**Mn**	-0.64	-0.67	-0.76	-0.41	-0.66	-0.47	-0.63	-0.62	-0.75	0.10	-0.26	-0.15	-0.05	0.02	-0.79	-0.16	-1.12	0.60	0.49	1.86	-0.52	-1.17	0.10	0.32
2010	**Ni**	-1.23	-1.25	-1.79	-1.28	-1.20	-1.21	-1.22	-1.19	-1.47	-0.93	-1.38	-1.20	-0.63	-0.95	N.A	N.A	-1.01	-0.59	-0.22	-0.79	-0.48	-0.45	1.55	0.20
2009	**Ni**	-1.02	-1.49	-1.27	-1.21	-1.40	-1.64	-1.47	-1.52	-1.37	-1.29	-1.17	-1.46	-0.92	-1.18	-0.32	-1.21	-1.65	-0.79	-0.69	-0.95	-0.56	-0.25	-0.76	0.17
2010	**Pb**	1.95	1.73	1.57	1.58	1.51	2.15	1.95	2.30	2.03	1.39	1.25	2.00	2.41	2.05	N.A	N.A	2.74	2.99	2.37	2.63	2.60	2.91	5.45	3.91
2009	**Pb**	2.92	3.18	3.18	3.27	3.58	4.16	3.84	3.72	3.33	2.19	2.95	3.00	2.80	3.04	3.38	2.57	4.57	4.57	3.38	4.40	4.12	4.82	3.36	6.02
2010	**Zn**	2.38	2.20	1.33	1.66	1.73	2.01	2.05	2.32	2.03	1.90	1.18	2.48	2.56	2.46	N.A	N.A	2.67	3.20	3.16	2.64	2.61	3.25	5.13	4.33
2009	**Zn**	1.65	1.76	1.65	1.92	1.81	2.13	2.00	1.87	1.56	0.77	1.51	2.06	1.26	1.74	1.98	1.07	2.48	3.31	2.26	2.34	2.20	3.14	1.83	3.74

Table 2 (continued). The same explanation as indicated in Table 2.

	I_{geo}	GA	CA	BA	ER	PO	RO	AS	AS2	SG	LU	KA	KA1	EL	DE1	DE2	DE3	ZO	ZO2	EU	SL	KT	AZ	PE1	PE2	PE3
2010	As	2.13	-1.24	-1.10	-0.41	-1.02	-0.48	-0.03	-1.14	-1.15	-0.98	-5.45	-3.67	-2.57	-1.74	-0.59	1.54	-2.75	-2.67	-4.10	-2.81	-8.31	-3.35	-5.74	-3.31	-3.84
2009	As	-0.51	-2.46	-1.66	-1.34	-1.05	-1.35	-0.95	0.57	-0.12	-2.73	-3.56	-4.66	-2.56	-2.57	-0.32	2.29	-2.25	-3.18	-4.33	-3.22	-3.58	-3.43	-3.51	-2.72	-2.19
2010	Cd	4.08	2.73	4.82	2.37	1.09	2.16	2.93	2.49	1.55	2.36	0.79	0.64	0.83	1.75	0.57	4.32	1.68	0.86	0.88	1.11	0.92	1.15	0.92	1.09	1.24
2009	Cd	3.26	0.08	4.57	1.26	1.17	1.12	2.19	3.19	2.26	0.55	-0.66	-1.30	0.23	0.27	0.13	3.14	0.87	-0.02	-0.62	-0.31	-0.60	-0.23	-0.49	-0.16	0.25
2010	Cr	-1.08	-1.95	-1.55	-2.21	-4.35	-2.41	-2.57	-3.02	-2.34	-2.17	-4.54	-5.39	-3.16	-2.04	-4.20	-1.06	-1.99	-2.34	-3.04	-1.93	-2.40	-2.07	-2.28	-1.36	-1.53
2009	Cr	-1.65	-3.67	-1.58	-2.63	-3.75	-2.89	-3.04	-2.28	-1.73	-2.55	-4.33	-5.01	-3.03	-2.68	-3.50	-2.63	-1.87	-2.70	-3.56	-3.17	-2.84	-2.06	-2.73	-1.87	-1.05
2010	Cu	2.78	2.13	2.16	1.12	0.21	1.45	1.31	0.35	-0.45	1.27	0.08	-1.04	-0.74	0.44	-1.39	2.63	0.49	-0.11	-1.03	0.04	-0.31	0.29	-0.04	0.39	0.43
2009	Cu	1.72	-0.14	1.78	0.11	0.71	-0.04	0.42	1.11	0.15	-0.28	-1.25	-1.95	-0.52	-0.43	-0.56	2.78	0.36	-0.59	-1.54	-0.79	-0.81	-0.38	-0.71	0.00	1.30
2010	Fe	-1.05	-1.59	-1.29	-1.62	-1.22	-1.66	-2.52	-2.65	-3.23	-1.97	-1.73	-2.40	-2.41	-1.90	-1.39	-0.50	-1.92	-2.00	-2.55	-2.11	-2.33	-2.09	-2.47	-2.31	-1.60
2009	Fe	-0.77	-1.81	-0.60	-1.02	-0.85	-1.67	-1.86	-1.98	-2.17	-1.72	-1.82	-1.96	-1.39	-1.67	-0.56	0.09	-1.48	-1.50	-2.01	-1.77	-1.88	-1.76	-1.89	-1.46	-0.71
2010	Mn	0.36	0.62	1.88	-1.04	-0.17	-0.97	-1.97	-1.55	-0.58	-1.17	-0.17	-0.62	-0.41	-0.41	-1.00	1.35	-1.19	-0.32	-0.56	-0.09	-0.61	-0.59	-0.60	-0.37	-0.22
2009	Mn	0.21	-0.01	2.73	0.15	1.28	-0.26	-0.94	-0.22	-0.13	-0.32	-0.12	-0.50	0.17	-0.10	-1.13	-1.09	-0.82	0.03	-0.42	-0.31	-0.50	0.05	-0.20	0.21	1.03
2010	Ni	0.94	-0.51	-0.15	-0.68	-1.19	-0.99	-1.19	-1.30	-1.25	-0.96	-2.00	-2.29	-1.23	-0.47	-1.70	0.03	-0.80	-0.72	-1.30	-0.50	-1.06	-0.76	-0.97	0.03	-0.23
2009	Ni	0.91	-2.02	-0.27	-1.08	-1.32	-1.36	-1.46	-0.82	-0.69	-1.14	-2.22	-2.87	-1.41	-1.11	-0.88	-0.39	-0.57	-1.18	-1.91	-1.51	-1.43	-0.56	-1.30	-0.43	0.17
2010	Pb	3.95	1.28	3.90	2.08	0.81	3.93	2.61	2.58	1.88	1.84	0.36	-0.05	0.11	0.94	-0.13	4.23	0.88	0.22	0.14	0.51	-0.04	0.34	0.10	0.62	0.67
2009	Pb	3.80	1.78	5.08	2.59	2.99	3.15	3.32	4.99	4.54	1.90	1.17	1.32	1.95	1.67	2.00	5.34	2.39	1.75	1.12	1.57	1.73	1.22	1.62	2.04	3.10
2010	Zn	3.87	2.24	4.53	2.15	0.64	1.98	1.92	1.45	0.56	1.82	0.63	-0.05	0.43	1.46	-0.14	4.21	1.14	0.79	0.26	0.99	0.63	1.03	0.48	0.78	1.00
2009	Zn	2.86	0.37	4.33	1.03	1.05	0.57	1.43	1.78	0.37	0.58	-0.27	-1.28	0.50	0.48	0.24	2.46	1.17	0.29	-0.21	0.11	-0.19	0.98	-0.06	0.20	0.86

Table 3. Comparison of the mean geoaccumulation indexes ($\overline{I_{geo}}$) calculated for the estuary of the Nerbioi-Ibaizabal River in 2010 with those calculated for other estuaries of the world: Quanzhou Bay (China), Western Part Egyptian Mediterranean Sea; Souani River (Gibraltar) [1, 10, 52]. The $\overline{I_{geo}}$ values are calculated as the average of the n I_{geo} values considered in each case. (N.A.: not available data).

	Samples (n)	***Extractant mixture***	***As***	***Cd***	***Cr***	***Cu***	***Fe***	***Ni***	***Mn***	***Pb***	**Zn**
Quanzhou Bay (China)	13	HNO_3:HCl: H_2O_2:HF	1.37	3.04	0.73	1.27	N.A.	1.22	N.A.	0.75	0.22
Egyptian Mediterranean Sea	8	HF:$HClO_4$:HNO_3	N.A.	1.27	-0.55	-0.05	-4.12	0.36	-3.63	-0.36	-0.75
Souani River (Gibraltar)	7	H_2O_2 :HCl: HNO_3	N.A.	0.46	-1.54	-1.43	1.57	-2.26	-1.80	-0.16	-0.97
Nerbioi-Ibaizabal 2010	49	HNO_3: HCl	-0.96	1.95	-2.21	1.28	-1.85	-0.49	-0.85	1.81	1.92

The geoaccumulation indexes go further that the previously described normalized average weighted concentrations. They provide us with very interesting information about the quality of sediments of a specific emplacement (contamination level and distribution pattern of contamination), and allow us comparing the results with other environments. They must be handled with care, however, because their significance depends on the reliability of the background values used in the calculation. Sometimes, background values are simply not available and their estimation is a long and expensive process. The I_{geo} values are only comparable, in addition, if comparable concentrations (acid leachable fraction, total concentration…) are used in their calculation, a condition which is difficult to full fill using bibliographic data (see Table 3). In any case, the most important limitation of the geoaccumulation index is that they do not provide any information about the actual toxicity of sediments. Even if a pollutant concentration is well above its background value, there is no matter of concern if the pollutant is not available to living organisms and, consequently, produces no toxic effect on them.

3.3. Classification of sampling sites according to Sediment Quality Guides (SQGs)

The Sediment Quality Guidelines (SQG's) express the relation between chemicals available in sediment and the adverse effects they cause on benthic communities. They refer to individual pollutants. The use of mean sediment quality guidelines quotients (mSQGq) has been proposed to account simultaneously for the combined effect of different pollutants present in the sediment [28]. The mSQGq's have been broadly used in different parts of the world [53-56]. They are calculated by dividing the pollutant concentration measured in sediment by its respective SQG to obtain the corresponding sediment quality guideline quotient (SQGq), then summing the resulting SQGq's (as much SQGq's as pollutants considered) and finally dividing by the number of pollutants considered. The effects range-median (ERM) has been the most widely used SQG to calculate the corresponding mERMq of sediments [15, 28, 29, 32, 57-60]. The ERMs represent midrange concentrations of chemicals above which adverse effects on a wide variety of benthic organisms are likely to occur [30]. ERM values have been reported for only 7 of the 14 elements considered in this work (As, Cd, Cr, Cu, Pb, Ni and Zn). The other elements are considered to be non-toxic, or toxic only at extremely high concentrations. The mERMq's have been compared to the probability of toxicity observed in experiments carried out in sediments. These experiments are specific laboratory tests done in order to study the survival percentages of amphipods after the application of different ranges of chemical concentrations [61]. According to the results obtained, mERMq values in the range 0-0.1, 0.1-0.5, 0.5-1.5, and >1.5 indicate a probability of being toxic of, 9% (non-toxic), 21% (slightly toxic), 49% (moderately toxic) and 76% (highly toxic), respectively.

The mERMq values of the sediments collected in the estuary of the *Nerbioi-Ibaizabal* estuary have been computed in this work, and the results obtained are shown in Figure 4. The sites with the highest probability of toxicity in 2009 were *BA* and *AX2*, with mERMq values of 1.65 and 2.24 respectively.

Moderately toxic sediments were found, in addition, in all the area of influence of the Gobela tributary River – including the sites located in its bed (*GO*, *GO1*, *GO2* and *EX*), the Lamiako sampling point (*LA*) and the Udondo semi-closed dock (*UD*) –, the right side of the Abra Bay – especially in the surroundings of two little leisure harbours (*5-8*, *AR*) –, the upper part of the Asua tributary (*AS2* and *SG*), the site immediately downstream the WWTP (*GA*) and the most inner part of the Deustu closed channel (*DE3*).

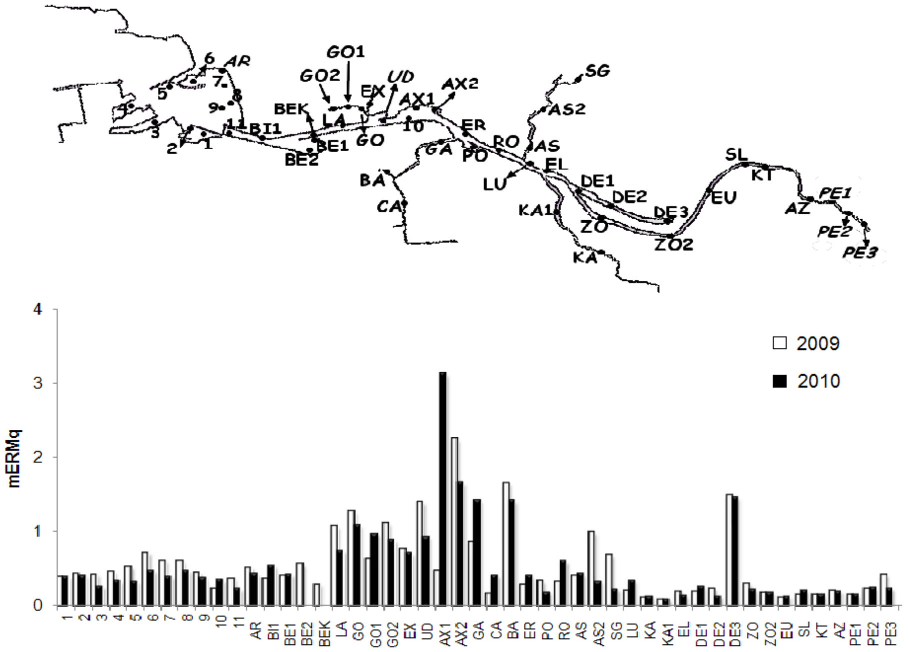


Figure 4. Mean Effects Range-Median quotient (mERMq) values calculated for each sampling site of the estuary in January 2009 and January 2010.

The rest of sediments were catalogued as not toxic or slightly toxic.

Broadly speaking, a slight decrease in toxicity of sediments is observed in 2010, but the sites of most concern are similar to those identified in 2009. In 2010, highly toxic sediments concentrate in the Axpe semi-closed dock (*AX1* and *AX2*). The area of the Gobela (*GO*, *GO1*, *GO2, EX*, *UD* and *LA*) and Galindo (*BA* and *GA*) Rivers still present sediments with moderate toxicity. The inner part of the Deustu channel (*DE3*) also remains as moderately toxic. Sediments of the rest of the sites, including those of the Abra Bay, are non-toxic or slightly toxic.

In spite of the common and easy use of the mSQGs, there are some assumptions in their calculation that should be taken into account for the correct interpretation of the results obtained after their application. When dividing the concentrations of different chemicals by their respective SQG and then summing and calculating the mean with all of them for each sampling site, we assume that the contributions of chemicals to toxicity are additive [62], and this is not always true. We also assume that all the chemicals under study have the same mechanism of toxicity in different living organisms, and that there is no synergic effect among pollutants. In few words, we trust that results obtained in controlled laboratory experiments are applicable to real conditions which considerable vary from environment to environment.

3.4. Complementary interpretation of the NAWC, I_{geo} and mERMq values calculated for the estuary of the Nerbioi-Ibaizabal River

A summary of the results obtained after the calculation of the Normalized Average Weighted Concentrations (NAWC), the geoaccumulation indexes (I_{geo}) and the mean Effects Range-Median quotients (mERMq) is shown in Table 4 for the sediments collected in 2009. The NAWC and mERMq approaches give us broadly the same situation in terms of spatial distribution of pollution in the estuary. The most polluted area is identified in the middle estuary, between the Asua tributary and the Lamiako sampling point, together with the most inner part of the Deusto channel. A second, but significantly lower, area of concern is the Abra Bay, especially in the surroundings of the sport harbours at the right side of the Bay. The I_{geo} approach allows us identifying specific element contamination in each sampling site. Cd and especially Pb are quite homogeneously present from the Abra Bay to the mouth of the Asua tributary. In addition, the middle estuary and the most inner part of the Deustu channel (*DE3*) are also seriously affected by Cu, Zn and As (*BA* also by Mn). It should be underlined that although many sites presented I_{geo} values higher than 4 (from highly to very highly polluted zone) for elements such as Pb, Cd and Cu, only *AX2* and *BA* showed a 76% of probability for toxicity (highly toxic). Moreover, even though there are considerable amount of sites with I_{geo} values below 1 for most of the elements considered, only one of them (*KA1*) is classified as non-toxic by the mERMq approach.

4. Conclusions

The NAWC, I_{geo} and mERMq are very useful tools to investigate the extent and spatial distribution of pollution in estuarine sediments. The combined use of these indicators provides the decision maker with simple and easy-to-understand complementary information on the distribution pattern of contamination in a given environment. The information takes the form of very intuitive rates which allow classification of sediments according to their pollutant content and/or risk of toxicity.

The NAWCs and the mERMq's account for the simultaneous average effect of different pollutants. The former gives each chemical the same weight,

Table 4. Combined information of the NAWC, I_{geo} and mERMq values found for the sediments of the estuary of the Nerbioi-Ibaizabal River collected in January 2009. (N.A.: not available data. "–" :I_{geo} above three was not found).

Sites	***Igeo***	***Probability for toxicity according to mERMq***	***NAWC***	**Sites**	***Igeo***	***Probability for toxicity according to mERMq***	***NAWC***
1	3 (Cd, Pb)	21%	2.28	**CA**	-	21%	9.00
2	4 (Pb), 3 (Cd)	21%	2.43	**BA**	6 (Pb), 5 (Cd, Zn), 3 (Mn)	76%	1.86
3	4 (Pb), 3 (Cd)	21%	2.54	**ER**	3 (Pb)	21%	3.78
4	4 (Pb)	21%	2.96	**PO**	3 (Pb)	21%	1.07
5	4 (Pb), 3 (Cd)	49%	2.89	**RO**	4 (Pb)	21%	1.37
6	5 (Pb), 3 (Cd, Zn)	49%	4.32	**AS**	4 (Pb), 3 (Cd)	21%	2.91
7	4 (Pb), 3 (Cd)	49%	3.66	**AS2**	5 (Pb), 4 (Cd)	49%	1.85
8	4 (Cd, Pb)	49%	3.68	**SG**	5 (Pb), 3 (Cd)	49%	0.85
9	4 (Pb), 3 (Cd)	21%	2.59	**LU**	-	21%	0.40
10	3 (Pb, Cd, Mn, Zn)	21%	1.20	**KA**	-	21%	0.00
11	3 (Pb)	21%	1.75	**KA1**	-	9%	1.10
AR	5 (Cd), 3 (Pb, Zn)	49%	3.63	**EL**	-	21%	0.83
BI1	3 (Cd, Pb)	21%	2.34	**DE1**	-	21%	1.79
BE1	4 (Pb), 3 (Cd)	21%	2.21	**DE2**	3(Pb)	21%	7.61
BE2	4 (Pb), 3 (Cd)	49%	3.08	**DE3**	6(Pb), 4(Cd), 3 (As, Cu, Zn)	49%	1.05
BEK	3 (Pb)	21%	5.50	**ZO**	3 (Pb)	21%	0.69
LA	5 (Pb), 4 (Cd), 3 (As, Zn)	49%	7.25	**ZO2**	3 (Pb)	21%	0.11
GO	5 (Pb), 4 (Cd, Cu, Zn)	49%	4.89	**EU**	-	21%	0.37
GO1	4 (Cd, Pb), 3 (Cu, Zn)	49%	7.51	**SL**	-	21%	0.22
GO2	6 (Cd), 5 (Pb), 3 (Cu, Zn)	49%	3.95	**KT**	-	21%	0.77
EX	5 (Pb), 3 (Cd, Zn)	49%	7.43	**AZ**	3 (Cd)	21%	0.38
UD	5 (Pb), 4 (Cd, Zn), 3 (Cu, As)	49%	2.65	**PE1**		21%	1.10
AX1	4 (Pb)	49%	10.00	**PE2**	3 (Pb)	21%	2.67
AX2	7 (Pb), 4 (Cd, Cu, Zn)	76%	4.38	**PE3**	4 (Pb)	21%	1.31
GA	4 (Cd, Pb), 3 (Zn)	49%	0.60				

whatever its toxicity is, while the latter is somehow weighted according to the toxicity level of each pollutant.

The I_{geo} indexes, however, are specific for each pollutant. Both I_{geo} indexes and mERMq's allow comparison among different environments, while NAWCs are site-and time-specific. In other words, the NAWCs provide information at the local scale, the I_{geo} indexes give that information a wide world perspective using results exclusively from chemical analysis, and the mERMq's complement the picture with toxicological data. In any case, if comparison is to be made among different environments, special care must be taken so that the concentrations used in their calculations have been obtained under similar experimental conditions, and that both the reference background values and ERM values are reliable and representative of the studied areas.

The application of the above mentioned indicators to the study of metal and metalloid pollution in the estuary of the *Nerbioi-Ibaizabal* River according to the pollutant content of its sediments has allowed us to identify the middle part of the estuary as an area of concern, due to relatively high concentrations of Cd, As, Pb and Zn. Only the sediments of two specific sites, however, are catalogued as highly toxic.

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2. Atala

Datuen tratamendua: Multzoen identifikazioa

Multzoen identifikaziorako teknika kimiometrikoak

Azken hamarkadetan instrumentazio analitikoan eta ordenagailuen zientzian eman diren aurrerapenak direla eta, kimiometria ere esanguratsuki hazi da. 1992. urtetik aurrera, *International Chemometrics Society (ISC)* delako erakundearen sorkuntzaren ondoren, kimiometria kimikaz aparteko pisu handiko jakintza-gai bihurtu da.

XX. mendearen 70. hamarkadaren hasieran Svant Wold-ek eta Bruce R. Kowalski-k kimiometria kontzeptua aurkeztu zutenetik asko izan dira kimika analitikoaren alor honetarako proposatu diren definizioak. Arte edo trebetasun moduan definitu da, hots, analisi kimikoetan lortutako datuetatik informazio esanguratsua eta adierazgarria ateratzeko arte edo trebetasuna [1]. Definizio zehatzagoak ere egin dira: kimiometria matematikaren, estatistikaren, eta/edo metodo sinbolikoen eta grafikoen aplikazioa da, datu-matrizeetatik ahalik eta informazio gehien eskuratzeko [2]. Definizio gehienek, hala ere, kimiometriaren ondorengo abantaila hauek azpimarratzen dituzte:

1) datuetatik momentuko informazio erreala eskura daiteke era azkar batean,
2) jatorri ezezaguneko datu-multzoetatik informazio esanguratsua lortu daiteke,
3) dimentsio askotako datu-multzoetatik informazio argia eta zehatza eskura daiteke,
4) merkea da.

Laburbilduz esan daiteke, kimiometriaren bidez kalitate handiko informazioa lortu daitekeela modu azkar eta merke batean [3].

Kimiometriaren aplikazioak ugariak dira [4]. Atal honetan aldagai anitzeko metodoen barnean sailkatzen diren multzoen identifikaziorako (*Pattern recognition*) tekniketan sakonduko da.

Kimikoki karakterizatu diren objektuen (laginen) arteko erlazioak identifikatzea ohiko arazoa izaten da Kimika Analitikoaren arloan. Multzoen identifikaziorako teknikek laginen artean antzeko ezaugarriak dituzten taldeak badaudela ontzat ematen dute eta antzekotasunak oinarritzat hartzen dituzte laginen sailkapena edo multzokatzea burutzeko [5]. Watanabe-k joera horiek *kaos*-aren aurkari moduan definitu zituen [6]. Multzoen identifikaziorako teknikek dokumentuen klasifikazioa, biometrika, finantza–iragarpena eta hizkeren identifikazioa dute lehen aplikazio-eremu [7]. Teknika guzti hauek oinarrizko hiru urrats biltzen dituzte: 1) datuen tratamendu matematikoa; ii) datuen errepresentazioa grafikoa eta, azkenik, iii) objektuen klasifikaziorako erabakia hartzea [7].

Datu-matrizea osatzen duten laginei buruz eskura dagoen, edo erabili nahi den, informazioaren arabera, multzoen identifikaziorako ikuskatutako (*supervised*) edo ikuskatugabeko (*unsupervised*) teknikak daude. Lehen kategorian, laginen azpitalde batean (*training set* delakoan) laginak multzokatzearen arrazoia edo ezaugarria ezaguna da, eta horretan oinarritzen da ikuskatutako teknika lagin berriak multzo desberdinetan sailkatzeko. Ikuskatugabeko azterketetan, ordea, sailkapena laginen arteko antzekotasunak edo desberdintasunak kontutan hartuz egiten da soilik, laginei buruzko aldez aurretiko inolako informazio gehigarririk erabili gabe.

Multzoen identifikaziorako ikuskatutako teknikak iturri desberdineko datu kimikoen azterketan erabiltzen dira, besteak beste, kromatografikoak, espektroskopikoak, eta sentsorialak [8]. Ikuskatutako teknika ugari daude. Kasu bakoitzean egokiena eskuen artean dugun arazoaren araberakoa da, tekniken oinarriak eta irispenak esanguratsuki aldatzen baitira batetik bestera. Irizpide desberdinak jarrai daitezke ikuskatutako tekniken sailkapenerako, hona hemen erabilienak:

- *Teknika parametrikoak/ez-parametrikoak*. Teknika parametrikoek parametro doigarriak dituzten eredu matematikoak erabiltzen dituzte laginen sailkapena burutzeko. Hauen artean LDA (*Linear Discriminant Analysis*), SIMCA (*Soft Independent Modelling of Class Analogy*), UNEQ (*UNEQual dispersed classes*) eta PLS-DA (*Partial Least Squares Discriminant Analysis*) daude. Teknika ez-parametrikoek ez dute parametrodun eredu matematikorik erabiltzen laginen sailkapenean, eta ezagunenen artean kNN (*k-Nearest Neighbours*), ANN (*Artificial Neural Networks*) eta CAIMAN (*Classification And Influence Matrix ANalysis*) aipa daitezke [9].

- *Teknika zorrotzak (discriminant)/bigunak (class-modelling).* Multzoen identifikaziorako ikuskatutako teknikek laginak karakterizatzen dituzten aldagaien hiperespazioa eremu desberdinetan banatzen dute. Teknika zorrotzen kasuan lagin berri bat eremu horietako batean kokatzen bada, eremu horri dagokion motakoa (klasekoa) da eta kanpoan kokatuz gero, berriz, ez da mota (klase) horretakoa; tarteko egoerarik ez dago. Metodo hauen artean LDA, kNN, PLS-DA eta ANN aipa daitezke. Teknika bigunen kasuan laginen klasifikazioa ez da hain mugatua, eta hiru posibilitate eman daitezke: objektua i) klase bati dagokion eremuan kokatzea; ii) bi eremuen artean kokatzea eta iii) definituriko eremu guztietatik kanpo kokatzea. Teknika hauen artean SIMCA eta UNEQ dira erabilienak [10].

- *Teknika deterministak/probabilistikoak*. Teknika deterministek lagin bakoitza klase batean sailkatzen dutenean ez dute erabaki honen fidagarritasuna adierazten. Probabilistikoek, ordea, egindako klasifikazioaren fidagarritasunaren estimazioa egiten dute. Lehenengoen artean kNN, eta CAIMAN daude eta probabilistikoen artean LDA, PLS-DA, SIMCA, UNEQ eta ANN.

Baina, askotan, laginak multzokatzeko eredua edo arrazoia ez da aldez aurretik ezagutzen. Egoera hau arrunta izaten da kutsadura monitorizatzeko ikerketetan. Kasu honetan, eta beste batzuetan, multzoen identifikaziorako ikuskatugabeko teknikak erabiltzen dira.

Ikuskatugabeko teknikak hiru multzo nagusitan bana daitezke:

i) Mordoen analisia (Cluster Analysis, CA): Hau da orain dela urte batzuk arte multzoen identifikazioan nagusiki erabiltzen zen metodoa. Teknika hau objektuak karakterizatzeko erabili diren aldagaien (propietateen) antzekotasun-mailan oinarritzen da antzekoak diren laginak mordo berean multzokatzeko eta, aldi berean, antzekoak ez diren laginak mordo desberdinetan multzokatzeko. Mordoen analisirako metodo ugari dauden arren, denen oinarria berdina da [11].

ii) Neurona-sare artifizialak (Artificial Neural Networks, ANN): multzoen identifikaziorako ereduen eraketan gizakion nerbio-sistema imitatzen duten teknikak dira eta oso eraginkorrak izaten dira sailkapen-prozesuan sarri ematen diren zailtasunei aurre egiteko. ANN-ak sarrerako datu-multzo batetik (*training set* delakotik) abiatzen dira, lagin desberdinetan neurtutako ezaugarri batzuk (kontzentrazioak edo espektroak, adibidez) izaten direnak, laginek multzo baten kide izateko duten probabilitatea kalkulatzeko (irteerako aldagaiak). Sare artifizialak ikuskatutako zein ikuskatugabeko multzoen identifikazioan erabili daitezke [12].

iii) Aldagai nagusitan (Principal Components, PC, edo Eigenvector delakoetan) oinarritutako metodoak: metodo hauen helburu nagusia objektuei buruzko *n* dimentsioko informazioa bi edo hiru dimentsiotara mugatzea da. Era honetan lagin bakoitza grafikoki adieraz daiteke bi edo hiru dimentsiotako espazio batean, joera nagusien identifikazioa errazten duena. Laginei buruzko informazioaren antolamenduaren arabera multzoen identifikaziorako teknika desberdinak bereiz daitezke. Laginak norabide batean eta aldagaiak beste batean kokatuz gero, bi dimentsiotako datu-matrizea lortzen da. Aldagaiak, adibidez, laginen osagaien kontzentrazioak edo espektroak izan daitezke. Datuen antolamendu hori erakusten duten metodoak bi dimentsiotako metodo moduan ezagutzen dira. Hauen artean osagai nagusien bidezko analisia (PCA, *Principal Component Analysis*) eta MCR (*Multivariate Curve Resolution*) dira nagusitu direnak. Bi dimentsiotako tekniken artean sarri aplikatzen diren beste batzuk FA (*Factor Analysis*), TTFA (*Target Transformation Factor Analysis*) eta PMF (*Possitive Matrix Factorization*) dira [13]. Baina askotan hiru dimentsiotako antolamenduak erabiltzen dira. Kasu honetan laginak espazio eta denboraren arabera antolatzen dira bi dimentsio desberdinetan, eta aldagaiak hirugarren dimentsioan kokatzen dira. Hiru dimentsiotako metodoen artean erabilienak PARAFAC (*PARallel FACtor Analysis*) eta Tucker3 dira [14], STATIS (*Structuration des Tableaux A Trois Indices de la Statistique*) eta PARAFAC2 ere sarri askotan aplikatzen direlarik.

PCA datu-matrize handien dimentsioa murrizteko teknika erabilienetarikoa da. Matematikoki, PCA-k neurtutako aldagaien dispertsioa biltzen duen kobariantza matrizean oinarritzen da “eigen-balioak” eta “eigen-bektoreak” kalkulatzeko. Jatorrizko aldagaien konbinazio linealaren ondorioz elkarren artean ortogonalak diren Osagai Nagusiak (*Principal Components*) kalkulatzen dira. Osagai nagusiek hasierako datuen bariantza osoa biltzen dute eta, ondorioz, laginen banaketa baldintzatzen dute [15]. MCR-k ere bi dimentsiotako matrizetan oinarritu arren, PCA-ren Osagai Nagusien baliokideak diren Osagaiak (*Components*) kalkulatzeko aplikaturiko mugak naturalagoak dira, hau da, kasu honetan eraturiko Osagaiak ez dira zertan ortogonalak izan behar. Honen ordez, negatibotasun eza bezalako murrizketa-teknikak aplikatzen dira, askotan aztergai diren aldagaiek (kontzentrazioek edo seinale espektralek, esaterako) balio negatiboak izateak zentzurik ez baitauka. Metodo honen zailtasuna emaitzen interpretazioan datza, ez delako erantzun bakarra lortzen, PCA-n gertatzen den bezala [16].

PARAFAC-en bidez, aztergai den hiru dimentsiotako datu-matrizea aldagaiez (*loading*-ez) osatutako *mode* izeneko hiru matrize berrietan deskonposatzen da eta hauek osagai (*component*) kopuru berdina dute. *Mode* hauen osagaiak *G core array* izeneko matrizearen bidez daude erlazionatuta. *G core array* delako matrizeak bat zenbakiaz osatutako super-diagonala duenez (matrizearen beste osagaiak zero dira), *mode* izeneko hiru matrizeen arteko erlazioa zuzena da. *Mode*-en osagaien errotaziorako askatasun-falta dela eta, PARAFAC-en bidezko analisietan lortzen den soluzioa, laginen banaketari dagokionez, bakarra da, emaitzen interpretazioa errazten duena [17]. Tucker3-ren bidez lortutako emaitzak, ordea, interpretatzeko zailagoak izaten dira. Kasu honetan *mode* matrize bakoitzak osagai-kopuru desberdina izan dezake eta osagaiak elkar erlazionatzen dituen *G core array* delakoa zero eta bat zenbakiez osatuta ez dagoenez, *mode*-en osagaien arteko erlazioa ez da zuzena. Gainera, *mode*-en osagaiak ortogonalak ez direnez, hau da, errotaziorako askatasuna dutenez, Tucker3-ak eskaintzen duen soluzioa ez da bakarra [18].

Multzoen identifikaziorako tekniken (eta batez ere Osagai Nagusitan oinarritutako tekniken) erabilera, zientzia-arlo askotara (degradazio-prozesuen zinetikara, metabolomikara, sentsoreen erantzunen azterketara eta irudien analisira, esaterako [19-22] zabaldu diren arren, azken aldian ingurumeneko laginen analisiaren esparruan izan duten gorakada da aipagarria. Hau inguru natural desberdinetan ezarritako araudi zorrotzen ondorio zuzena dela pentsa daiteke. Ur-masen kasuan, esaterako, multzoen identifikaziorako tekniken aplikazioak etengabe egin du gora 2000. urtean Europako Uraren Zuzentaraua (*European Water Framework Directive,* WFD, 2000/60/EC) ezarri zenetik. WFD delakoaren helburuak betetzeko bidean aurrera eramaten diren monitorizazio-saioetan interpretatu beharreko aldagai anitzeko datu-sorta luzeak sortzen dira. Monitorizazio-saio hauetan, normalean, laginketa-kanpaina, -zonalde eta lagin mota ugari kontutan hartzen dira aldi berean, konplexutasun eta aldakortasun handiko datu-sorta luzeak sortuz [23]. Elkar erlazionaturiko informazioa dutenez, datu-sorta hauen analisian aldagai bakarreko edo dimentsio bakarreko ereduak erabiltzeak ez du zentzurik, bidean informazio ugari galduko litzatekeelako [24]. Kutsadura-iturrien ikerketan, esaterako, aldagai bakarreko ereduak aplikatzean, kutsadura-iturri bakarra dagoela ontzat ematen da aldez aurretik, hau da, puntu desberdinetan harturiko laginen kutsatzaileen arteko erlazioa lineala dela ontzat ematen da, kasu gehienetan egia ez dena [25].

Tesi honen aztergaia itsasadarretan metalen bidezko kutsadura ikertzea denez, arlo honetako multzoen identifikaziorako tekniken aplikaziorik garrantzitsuenak laburbilduko dira jarraian. Espero zitekeenez, argitaratutako lan gehienak azken hamarkadakoak dira, kimiometria XX. mendearen 70. hamarkadan garatu zen arren, ingurumeneko aplikazioak nabarmenki beranduago heldu baitziren.

Laburbilduz esan daiteke multzoen identifikaziorako lan gehienak lurzorutan eta sedimentutan egin direla. Lurzorutan egindako lanen laburpen ona argitaratu du Mostert-ek orain dela gutxi [26]. Hauen atzetik uretan eta airean egindako azterketak [27, 28] kokatzen dira eta, distantzia handiz, biotan burututakoak [29, 30]. Aplikaturiko tekniken artean, mordoen analisia (CA) eta osagai nagusien analisia (PCA) dira nabarmendu direnak. Hala ere, hiru dimentsiotako tekniken erabilera, PARAFAC-ena eta Tucker3-arena nagusiki, hedatzen hasi da azken aldian.

Multzoen identifikaziorako teknikak erabiltzean helburu desberdinak aipatzen dira. Kutsadura-iturrien identifikazioa konpartimendu desberdinetan (uretan, sedimentuetan, airean, eta abarretan) gehien aipatzen den horietariko bat da. Lan-ildo honetan PCA eta CA dira nagusitu direnak [27, 31-35], baina hiru dimentsiotako Tucker3 eta PARAFAC [28, 36-38], eta *Kohonen Neural Networks* delakoak, KNN-ak [33, 39] ere aplikatu dira. Sarri proposatu den beste helburu bat kutsaduraren denborarekiko eta espazioarekiko aldakortasunaren azterketa izan da. Honetan ere, gehien erabilitako teknikak PCA eta CA izan dira [35, 40-43]. Baina hiru dimentsiotako teknikak erabiliz ere zenbait lan egin dira: Tucker3 soilik erabiliz [44] edo teknika hauen emaitzak PCA-k edo CA-k emandakoekin konparatuz [45]. Ingurumeneko konpartimendu desberdinen egoera ekologikoa aztertzeko ere multzoen identifikaziorako tekniken bidez lortutako distribuzioak eta sailkapenak informazio-iturri moduan erabili dira [23, 38, 46-51].

Aipatutako ohiko erabilera hauetaz aparte, multzoen identifikaziorako teknikak bestelako informazioa eskuratzeko ere aplikatu dira. Esaterako, erreminta kimiometriko berrien emaitzen egokitasuna berresteko. Adibidez, PCA-ren aurretik burutu beharreko datuen aurretratamenduan erabil daitezkeen teknika desberdinen eraginkortasuna aztertu da, sedimentuetako datu-sortak erabiliz [52]. MOLMAP (*MOlecuLar Maps of Atom-level Properties*) izeneko multzoen identifikaziorako teknika berri baten erabilgarritasuna ere frogatu zen, lortutako emaitzak PARAFAC eta MA-PCA-aren (*Matrix-Augmented Principal Components Analysis* delakoaren) bidez lortutako emaitzekin konparatuz [53]. Osagai Nagusien bidezko analisian oinarritutako beste teknika berri bat, hots, *fuzzy PCA* delakoa ere aurkeztu da, lurzorutako kutsadura metalikoaren distribuzio espaziala aztertuz [54]. Antzeko bidea jarraituz, hiru dimentsiotako *STATIS* delako teknikaren aplikagarritasuna ere behatu da lurzoru laginak erabiliz [55].

Sedimentutan eta lurzorutan biltegiraturiko metalen eskuragarritasuna eta espeziazioa aztertzeko lanak ere aipagarriak dira. Erauzketa sinpleen eta sekuentzialen bidez lortutako kontzentrazio metalikoak biltzen dituzten datu-matrizeak multzoen identifikaziorako tekniken bidez analizatu dira, joera esanguratsuenak identifikatu nahian [40, 56-58]. 2011an egindako laburpen-lan batean, kimiometriaren papera aztertzen da erauzketa sinple eta sekuentzialen lan-eremuan [59]. Bukatzeko aipatu behar da, ikertutako konpartimenduak asko izan diren arren, lan gehienetan konpartimendu bakarra aztertu dela. Gutxi dira multzoen identifikazioa konpartimendu desberdinetara hedatzera ausartu direnak [30], beste arrazoien artean, honek dakartzan dimentsio-zailtasunak direla eta.

Tesi honen aztergai den Nerbioi-Ibaizabal itsasadarrean ur- eta sedimentu-laginak bildu diren arren, kutsatzaileen biltegi nagusiak diren sedimentuak soilik hartu dira kontutan multzoen identifikaziorako teknikak aplikatzeko orduan. Ur-laginek momentu eta espazio zehatz bateko informazio puntuala besterik ez dute eskaintzen. Sedimentuak, ordea, metalen biltegi moduan jokatzeaz gain, kutsadura-iturri ere bilakatu daitezke [60]. Garrantzitsua da, beraz, sedimentuetan bilduriko metalen kontzentrazioen joerak, bai denboraren zein espazioaren araberakoak, identifikatzea eta ikertzea. Era honetan, itsasadarraren egoera ekologikoa aztertu eta balizko kutsadura-iturriak identifikatu ere egin daitezke. Hurrengo atalean, ondorioz, Nerbioi-Ibazabal itsasadarreko laginketa-puntu eta -kanpaina desberdinetan bildutako sedimentuetan neurtutako traza-mailako elementuen joerak identifikatzeko saioa deskribatzen da. Multzoen identifikaziorako teknikak erabili dira helburua betetzeko, alde batetik, bi dimentsiotakoak (PCA eta MCR) eta, beste aldetik, hiru dimentsiotakoak (PARAFAC eta Tucker3). Teknika bakoitzaren bidez lortutako emaitzak interpretatu dira ingurumeneko terminotan. Tekniken arteko konparaketa kritikoa ere egin da teknika erabiltzeko erosotasuna, emaitzen interpretazioaren erraztasuna eta emaitzek emandako informazioaren kantitatea eta kalitatea bezalako gaiak eztabaidatuz.

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Feasible comparison among multivariate chemometric techniques for pattern recognition used in environmental monitoring

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Environmental Pollution **aldizkari zientifikora bidalia.**

Feasible comparison among multivariate chemometric techniques for pattern recognition used in environmental monitoring

Abstract

Environmental datasets usually contain multiple and highly correlated variables. The use of multivariate techniques in this case has experienced a significant development. This paper provides a feasible comparison among several methods for pattern recognition: Principal Component Analysis (PCA), Multivariate Curve Resolution (MCR), Parallel Factor Analysis (PARAFAC) and Tucker3. They have been systematically applied on the same dataset, consisting on the concentration of trace elements measured in sediments of the estuary of the *Nerbioi-Ibaizabal* River (Basque Country). The results obtained have been critically discussed in terms of easy of use, interpretation of the results, and amount of information provided. The most popular PCA is probably enough for a correct and overall interpretation of the results. MCR, however, may lead to slightly different results due to the non-perceptive orthogonality of the principal components. Both PARAFAC and Tucker3 provide simplified graphical outputs that make easier the interpretation of the results.

Keywords: Sediments; Metals; Multivariate analysis; Two- and three-way models; Estuary of Bilbao.

1. Introduction

Environmental monitoring datasets usually contain highly correlated information, so that the use of univariate methods is not enough for the exhaustive extraction of relevant information (Alvarez-Guerra et al., 2010a). Moreover, the development in the last decades of very fast analytical instrumentation able to measure simultaneously the concentration of a considerable number of analytes per sample has also promoted the creation of large experimental datasets (Reid and Spencer, 2009). In this situation, methods for multivariate analysis of data are valuable tools, due to their ability to distinguish significant trends and sources of variation, commonly difficult to see using more classical approaches (Qishlaqi et al., 2009). The resulting models can also be depicted in intuitive plots, providing us with an easy and quick visualization of the relations among objects and variables contained in the environmental dataset (Mas et al., 2010; Peré-Trepat et al., 2007). Consequently, not only pattern recognition issues, but also classification and prediction ones, are more often attempted by multivariate chemometric tools in the analysis of large amounts of environmental data (Alvarez-Guerra et al., 2010a) (see some important references in Table 1) (Abollino et al., 2002; Alvarez-Guerra et al., 2010b; Cukrowska et al., 2004; Fernández et al., 2008; Gong et al., 2005; Latorre et al., 1999; Martuzevicius et al., 2008; Peré-Trepat et al., 2007; Ratola et al., 2010a; Ratola et al., 2010b; Razic et al., 2005; Simeonov et al., 2007; Simeonova and Simeonov, 2006; Singh et al., 2005; Skrbic and Durisic-Mladenovic, 2010; Stanimirova et al., 2004; Stanimirova et al., 2006; Terrado et al., 2010).

When an environmental study consists, for instance, of only a single-time campaign, the data can be arranged in a simple two-way matrix (where samples are located in different rows and measured variables in the columns). However, environmental work is usually based on monitoring the sites under study over time, because both spatial and seasonal variations are expected to be important (Alvarez-Guerra et al., 2009; Navarro-Ortega et al., 2010). In pattern recognition, the resulting dataset can be analyzed in different ways, depending on the way the augmented data is created. A two-way arrangement of data (samples in one direction and variables in a second one) is appropriate for Principal Component Analysis (PCA) and Multivariate Curve Resolution (MCR), while a three-way arrangement of data (sampling points in one direction, sampling campaigns in a second one and variables in a third one) allows us to investigate the structure of the matrix by other chemometric methods such as Parallel Factor Analysis (PARAFAC) or Tucker3. The main features of the abovementioned methods are different, and so they are the results obtained by each one, in a way that the analysis of the same data matrix using different approaches usually provides us with complementary and enriching information about the area under study.

In this work we have compared the performance of the abovementioned methods to investigate the main sources of variation in an environmental dataset. The concentration of 14 elements in sediments collected in the estuary of the *Nerbioi-Ibaizabal* River (Bilbao, Basque Country) was selected as a case study. This area is located in the south-east corner of the Bay of Biscay and is

Table 1. Examples of long environmental monitoring datasets analysed by different multivariate methods.

Environmental compartment	**Analytes**	**Instrumental technique**	**Multivariate method**	**Reference**
Natural water	Physico chemical parameters pH, free acidity, total hardness, $\mathrm{Cl^-}$, $\mathrm{SO_4^{2}}$, $\mathrm{NO_3^-}$, $\mathrm{NO_2^-}$, dry residue, Ca, Mg, Na	Volumetric, potentiometric, atomic and molecular spectroscopic methods	PCA, MPCA, Tucker3	(Simeonova and Simeonov, 2006)
Fish, sediment and river water	As, Ba, Cd, Co, Cu, Cr, Fe, Mn, Ni, Pb, Zn	ICP-OES	PCA, MCR-ALS, PARAFAC, Tucker3	(Peré-Trepat et al., 2007)
Surface river water	Organophosphate compounds, triazines, propanil, chloroacetanilides, APs	HPLC	PCA, MCR-ALS	(Terrado et al., 2010)
Groundwater	Tributylphosphate, triazines, APs			
River sediment	PAHs, Organophosphate compounds			
Soil	PAHs			
Gold tailings dump material	Metals Ca, Mg, Fe, Mn, Al, Cr, Ni, Co, Zn, Cu Anions $\mathrm{NO_3^-}$, $\mathrm{Cl^-}$, $\mathrm{SO_4^{2-}}$	ICP-OES ITP analyzer	PFA	(Cukrowska et al., 2004)
Herbal Drugs	Cu, Zn, Mn, Fe, K, Ca, Mg, Al, Ba	FAAS, FAES, ICP-AES	PCA	(Razic et al., 2005)
Agricultural Soil	Al, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Ti, Zn	ICP-AES, GF-AAS	PCA, HAC	(Abollino et al., 2002)
Honey	Li, Rb, Na, K, Mg, Zn, Cu, Fe, Mn, Ni, Co	AAS, AES	PCA, LDA, KNN, SIMCA	(Latorre et al., 1999)
River ground and surface water	$\mathrm{NO_3^-}$, $\mathrm{Cl^-}$, $\mathrm{SO_4^{2}}$, $\mathrm{F^-}$, $\mathrm{PO_4^{3-}}$ K, Na, Ca, Mg, Cd, Cr, Cu, Fe, Mn, Pb, Zn, Ni	ICPF, AAS	DA, PCA, PLS	(Singh et al., 2005)
Soil	Cd, Cu, Cr, Ni, Pb, Zn	AAS	PCA, LDA	(Skrbic and Durisic-Mladenovic, 2010)
River and lake sediment	PCBs, PAHs Cr, Zn, Cu, Ni, V, Fe, Mn, Al, Li	GC-MS, ICP-AES, ET-AAS, HG-AAS, CV-AAS.	PCA, CA	(Simeonov et al., 2007)
Ginger oil	Volatile compounds	GC-MS	ELPG	(Gong et al., 2005)
River sediment and suspended matter	60 trace elements	TXRF, ICP-OES, ICP-MS, INAA	CA, FA	(Aulinger et al., 2002)
Soil depth profiles	Fe, Mn, Cd, Co, Cr, Cu, Ni, Pb, Zn	ICP-OES, ICP-MS	Tucker3	(Stanimirova et al., 2006)
Airborne particulate matter	38 trace elements	XRF	PARAFAC	(Martuzevicius et al., 2008)
Recompilation of bibliographic data	$\mathrm{H^+}$, $\mathrm{NH_4^+}$, $\mathrm{Na^+}$, $\mathrm{K^+}$, $\mathrm{Ca^{2+,}}$ $\mathrm{Mg^{2+,}}$ $\mathrm{Cl^-}$, $\mathrm{NO_3^-}$, $\mathrm{SO_4^{2-}}$	Non-specified	STATIS, Tucker3, PARAFAC 2	(Stanimirova et al.)
Estuarine water	As, As, Cr, Cu, Fe, Mn, Ni, Zn	ICP-MS	PCA	(Fernández et al., 2008)
Pine neddles	PAH	GC-MS	PCA	(Ratola et al., 2010a; Ratola et al., 2010b)
Sediment	As, Zn, Cd, Cr, Cu, Pb, Hg, Ni, Ag PAHs, p,p'-DDD, p,p'-DDE, PCBs, Hexachlorobenzene, p,p'-DDT	Non-specified	LDA, QDA, PLS-DA, ECVA, CART, CP-ANN	(Alvarez-Guerra et al., 2010b)

ICP-OES, inductively coupled plasma atomic emission spectroscopy; HPLC, high performance liquid chromatography; ITP, izotacophoresis; FAAS, flame atomic absorption spectroscopy; FAES, flame atomic emission spectroscopy; ICP-AES, inductively coupled plasma atomic emission spectroscopy; GF-AAS, graphite furnace atomic absorption spectroscopy; AAS, atomic absorption spectroscopy; AES, atomic emission spectroscopy; ICPF, ionic conducting polymer film; GC-MS, gas chromatography mass spectrometer; ET-AAS, electrothermal atomic absorption spectroscopy; HG-AAS, hydride generation atomic absorption spectroscopy; CV-AAS, cold vapour atomic absorption spectroscopy; TXRF, total reflection X-ray fluorescence spectrometry; INAA, instrumental neutron activation analysis; XRF, X-ray fluorescence.

MPCA, multi-way component analysis; SIMCA, soft independent modelling of class analogy; PFA, principal factor analysis; HAC, hierarchical ascending classification; LDA, linear discriminat analysis; kNN, Kth nearest neighbour; DA, discriminant analysis; PLS, partial least-squares, CA, cluster analysis, ELPG, evolving latent projection graph; FA, factor analysis; STATIS, structuration des Tableaux A Trois Indices de la statistique; QDA, quadratic discriminant analysis; PLS-DA, partial least squares-discriminant analisis; ECVA, extended canonical variates analysis; CART, classification regression trees; CP-ANN, counter-propagation artificial neural networks.

one of the most populated and important industrial areas in the Cantabrian coast (Barreiro and Aguirre, 2005; Cearreta et al., 2002). Sediments are considered essential to investigate the environmental health of coastal, estuary and river systems, due to its capacity to accumulate pollutants, such as trace metals (Alvarez-Guerra et al., 2010b; Álvarez et al., 2008; Moros et al., 2009; Rodríguez-Obeso et al., 2007). In this way the objectives of the study were twofold: i) to investigate if the combined use of two- and three-way multivariate methods provides us with more exhaustive environmental information than currently available (Bartolomé et al., 2006; Cearreta et al., 2002; Fdez-Ortiz de Vallejuelo et al., 2010a; Fernández et al., 2008; Moros et al., 2010; Raposo et al., 2008) on the specific system under study, and ii) to discuss critically, and under a practitioner point of view, the pro's and con's of each of the methods in terms of easy of operation, amount of information provided, and interpretation of the results.

2. Brief theoretical background

As previously stated, environmental data can be arranged in different ways. The data considered in this work has the general structure of several variables being measured at several sites over time. Hence, the data can be arranged in a three-way structure with sites in one direction (e.g. rows), variables in another (e.g. columns) and time in the third dimension (Figure. 1b). Nevertheless, the cube is often rearranged into a matrix/table. A simple way to do this is by placing the data from different sampling campaigns one on top of the other (data augmentation or column-wise augmentation), which is commonly used in two-way multivariate methods (Figure. 1a).

2.1. Two-way models

PCA and MCR methods, both of them used in this work can be defined as:

$$\mathbf{D_{aug}} = \mathbf{T}_{\text{aug}}\,\mathbf{P}^{\text{T}} + \mathbf{E}_{\text{aug}} \qquad (1)$$

where $\mathbf{D_{aug}}$ is the augmented two-way data matrix; $\mathbf{T}_{\text{aug}}$ is the score matrix; $\mathbf{P}^{\text{T}}$ is the loading matrix and the $\mathbf{E}_{\text{aug}}$ is the matrix of residuals. $\mathbf{P}^{\text{T}}$, in the case of an environmental dataset, contains information on the variables measured to characterise the samples, and $\mathbf{T}_{\text{aug}}$ is linked to geographical and spatial distribution of the studied area.

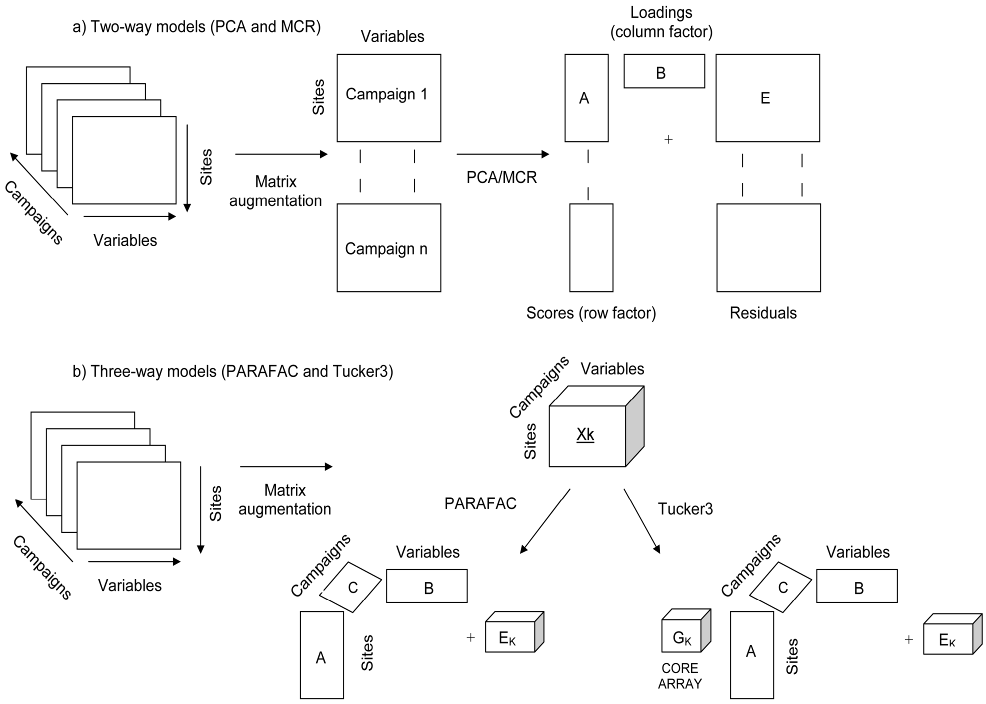


Figure. 1. Data matrix augmentation for the two-way chemometric methods, PCA, MCR-ALS (a), and the three-way chemometric methods, PARAFAC and Tucker3 (b).

Principal Components Analysis (PCA) is based on the decomposition of the original data matrix by calculating new (and few) variables (Principal Components) that are linear combination of the original ones. These variables are orthogonal to each other and describe the maximum sources of variance of the data (Amigo et al., 2010; Felipe-Sotelo et al., 2008). Nevertheless, the solution may be difficult to understand, because it is an abstract linear combination of the true experimental variance sources (Bin et al., 2004).

Contrary to PCA, MCR is intrinsically based on modelling spectroscopic data following a linear relationship between samples and variables (analogous to Beer Lamberts law), making it easier to interpret the model from an environmental point of view (Amigo and Ravn, 2009; Terrado et al., 2010). The loadings in MCR correspond to the underlying pure variables and the scores are the corresponding sampling sites.

An important difference is that while PCA factors (Principal Components) are restricted to be orthogonal, MCR does not impose orthogonality of the components (Mas et al., 2010; Pentti and Unto, 1994). Instead, the constraints commonly applied in environmental datasets are non-negativity of the scores and loadings (Tauler et al., 1998).

2.2. Three-way models

The main difference in the decomposition of the three-way dataset using a PARAFAC model or a Tucker3 model is principally the creation of the core array shown in the equations below:

$$\text{PARAFAC:} \quad \underline{\mathbf{X}}_{\underline{K}} = \mathbf{A}\mathbf{D}_{K}\mathbf{B}^{\mathrm{T}} + \mathbf{E}_{K}\,, \qquad K = 1,.., K \qquad (2)$$

$$\text{Tucker 3:} \quad \underline{\mathbf{X}}_{\underline{K}} = \mathbf{A}\mathbf{G}_{K}\mathbf{D}_{K}\mathbf{B}^{\mathrm{T}} + \mathbf{E}_{K}, \qquad K = 1,.., K \qquad (3)$$

where the *k*th slice of $\underline{\mathbf{X}}$ may refer to a sampling campaign. $\underline{\mathbf{X}}_{\underline{\mathbf{K}}}$ is a three-way data of size *I*×*J*×*K.* Here *I* is the number of sampling sites; *J* is the number of variables measured and *K* is the number of time points measured. **A**, $\mathbf{D}_K$ and $\mathbf{B}^{\mathrm{T}}$ reflect the first, second and third mode loadings respectively (the matrix **A** contains the loadings of the sampling sites, the $\mathbf{D}_K$ the loadings of the *k*th time and the matrix **B**, created with the loadings of the second mode, contains information on the variables measured to characterise the samples (trace element concentration in this case). $\mathbf{E}_K$ contains the residuals and the matrix $\mathbf{G}_K$ corresponds to the core array of the Tucker3 model (Smoliński et al., 2008).

A Tucker3 model has the capacity to extract different number of components from each mode and the components of the different modes are able to interact with each other. The three-way core array $\mathbf{G}_K$ is able to quantify these interactions (Evrim et al., 2008; Stanimirova and Simeonov, 2005).

Although Tucker3 allows visualization of the three-way data, the interpretation could be complicated (Stanimirova et al., 2004). The principal advantage of the PARAFAC model is the unique solution it is able to supply due to the lack of rotational freedom of the components on the different modes (Bro et al., 2010; Mortensen and Bro, 2006; Pravdova et al., 2002).

3. Experimental

3.1. Sampling and analysis

The samples were collected at eight different sampling sites along the estuary of the *Nerbioi-Ibaizabal* River (Figure 2); four of them are located in tributary rivers, Kadagua (*KA*), Galindo *(GA),* Asua *(AS)* and Gobela (*GO*), two of them in the main channel, Alde Zaharra (*AZ*) and Zorrotza, (*ZO*)*,* another one in a semi-closed dock*,* Udondo*, (UD),* and the last one in the mouth of the estuary, Arriluze (*AR*). Seventeen sampling campaigns were carried out from

January 2005 to April 2009, approximately every three months, so that 136 samples were collected and analysed.

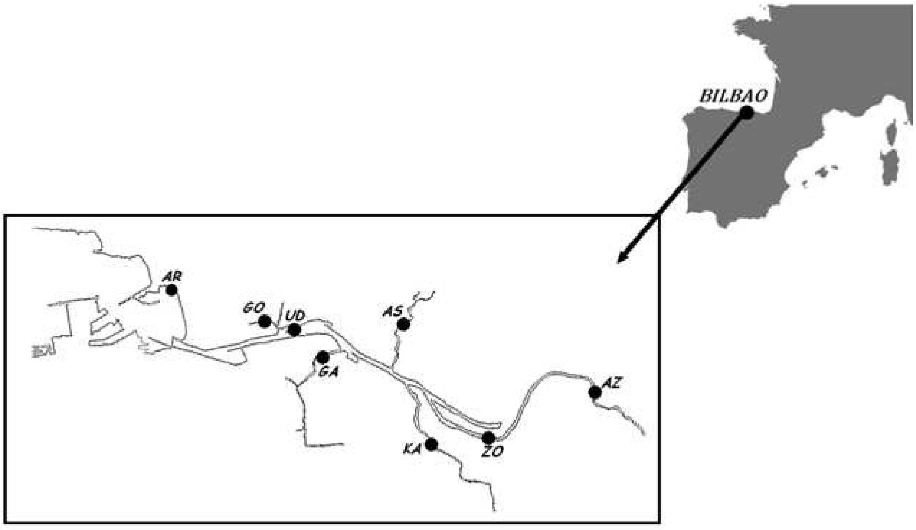


Figure 2. Sampling sites in the estuary of the *Nerbioi-Ibaizabal* River (Bilbao, Basque Country).

Only the surface sediment (0-5 cm) was collected and packed in plastic bags. The samples were transported to the laboratory at 4 ºC to reduce the microbiological activity. The sediments were frozen at -20 ºC, lyophilized at 150mTorr and -52 ºC for 48 h, and sieved. Only the fraction with a particle size lower than 63 µm was retained and analysed. The concentration of Al, As, Cd, Co, Cr, Cu, Fe, Mg, Mn, Ni, Pb, Sn, V and Zn was determined by ICP/MS, after extraction of the analytes in a the HNO_3/HCl mixture. Further details about the analytical procedure can be found elsewhere (Fdez-Ortiz de Vallejuelo et al., 2009).

A preliminary study of the structure of data using basic statistics has already been published (Fdez-Ortiz de Vallejuelo et al., 2010a). A similar data set has also been used to develop classification models using different chemometric approaches (Fdez-Ortiz de Vallejuelo et al., 2010b).

3.2. Data arrangement and data treatment

For the application of the two-way methods the data augmentation was performed in a column-wise manner (Figure 1a), and the dimensions of the augmented data matrix $\mathbf{D}_{\mathbf{aug}}$, were 136 × 14 (8 sites × 17 campaigns and 14 variables). The three-way methods were applied on a three way data array ($\underline{\mathbf{X}}$) (8 sites × 17 campaigns × 14 variables (Figure 1b)). Data analysis by PCA, MCR, PARAFAC and Tucker3 was performed using PLS-Toolbox (Eigenvector, Inc.), in MATLAB 7.0 (the Math Works, Inc). The problem of the missing data was handled using methods cited in the literature (Tomasi and Bro, 2005). The values below the detection limit (<LOD) were replaced by the half of the corresponding LOD (Zhang and O'Connor, 2005). Data was mean centred and scaled to unit standard deviation in the variables direction.

4. Results and discussion

4.1. Two-way models

4.1.1. PCA

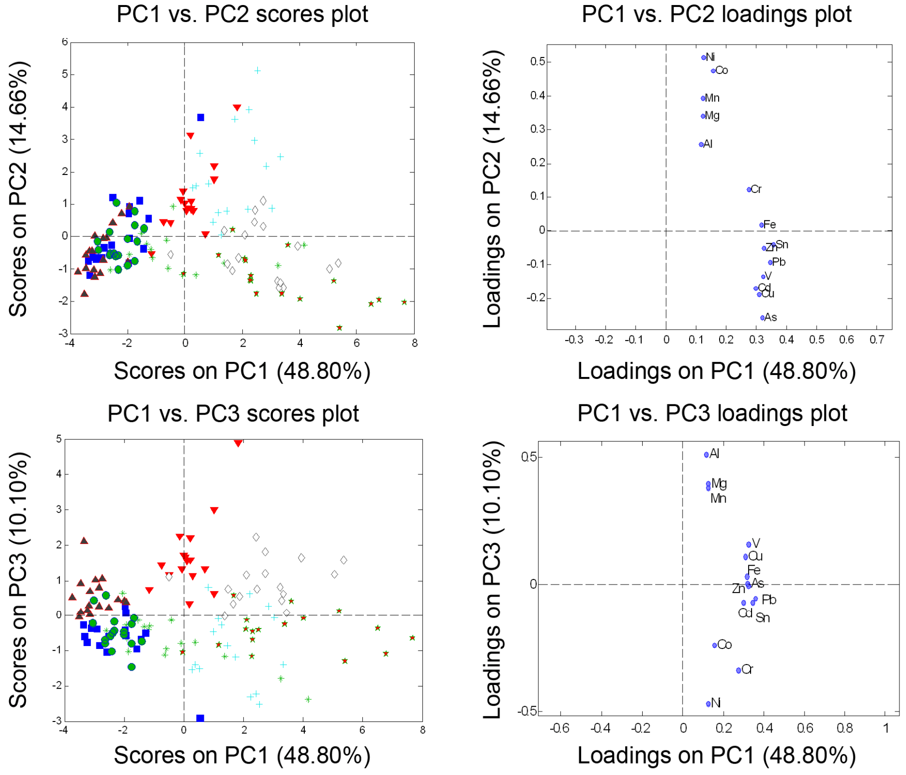
The first three PCs of the PCA model obtained explained the 74% of the variance, leaving the 26% of the original information in the residuals. Components explaining less than the 8% (PC4 explained only the 7.7%) of the data variance were not considered and were supposed to be due to data noise. Therefore, the three component model was finally selected.

Figure 3a shows the scores and loading plots over the PC1-PC2 and PC1-PC3 spaces. These plots suggest that the first component (49% of variance explained) is strongly related to the average element content of the sediments, in such a way that samples placed in its positive part have a higher average concentration of metals than those located in its negative part. Thus, the main source of variation in the dataset is the difference in average element concentration among sites.

The samples with the lowest scores for PC1 are from the *KA, ZO*, AS (with some exceptions) and *AZ* sites, which are located in the upper part of the estuary (see the map on Figure 2). There are some exceptions but, in general terms, those samples collected in the rest of the sites present intermediate (*AR* and *GA*) or high (*UD* and *GO*) score values for PC1.

The second (explaining 15% of the variance) and third (10% of variance) PCs primarily reflect which specific variables play a dominant role on the characteristics of each sample. PC2 is characterized by high loading values for Al, Mg, Co, Ni, and Mn. Some of these metals (e.g. Al, Mg), considering the composition of the basin, could be considered as natural. Nonetheless, the other metals certainly have an anthropogenic origin (Cearreta et al., 2000; Delgado et al., 2010).

a) PC1 vs. PC2 and PC3



b) PC1 vs. Sample plot

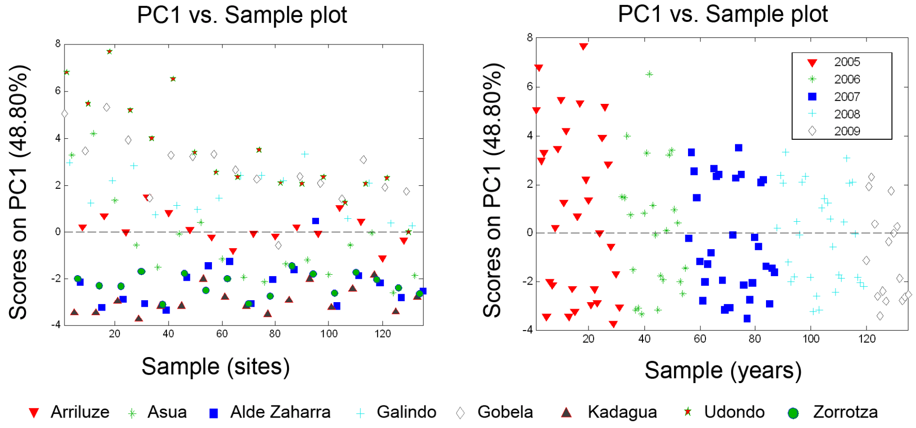


Figure 3. PCA results: scores and loadings plots on PC1-PC2, and PC1-PC3 (a); Plot of the scores on PC1 together with colour grouping of samples according to sampling site and sampling year (b).

Samples from *GA* and *AR* gather all together in the positive part of PC2, while those from *UD* and *GO* lay mainly in the opposite part. *GA* and *AR* sampling sites are geographically and geologically different: the first one is located downstream a sewage treatment plant located in the *Galindo* tributary River, and the second one is situated in the mouth of the estuary, near the open sea. On the other hand, *GO* and *UD* are geographically related: *UD* is located in a semi-closed dock, just before the point where the *Gobela* River joins the main flow of the estuary.

The third PC, however, helps us to differentiate, first, between *GA* and *AR* and, second, between *UD* and *GO*. *AR* is highly associated to elements like Al, Mg and Mn, while *GA* is more connected to anthropogenic Co, Ni and Cr. Similarly, *GO* is mainly influenced by elements like Cu, V and Fe, while *UD* is characterised by high concentrations of very toxic elements, such as Cd, Pb, Sn, Zn and As.

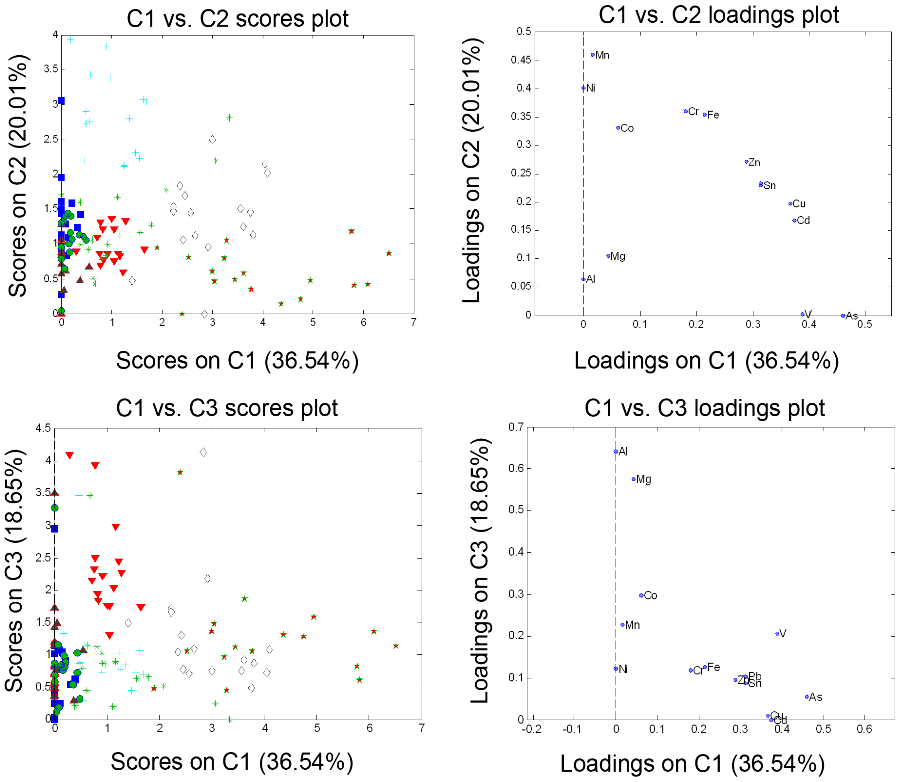
Sometimes, plotting the scores of the samples and identifying each sample by properties that characterise them, e. g., location or sampling year, provides further information usually hidden in the classical bi-plots (Figure 3b). For example, from Figure 3b, a slight decrease in average metallic concentration with time can be distinguished. This trend is especially noticeable in samples from the *AS*, *UD* and *GO* sampling points.

4.1.2. MCR

The model with three components, explaining more than 75% of the variance, was finally chosen, since the use of more components did not provide us with further significant information. Since the non-negative restriction was applied, all the samples presented positive score and loading values (Figure 4a).

The results obtained by MCR are, in general, very similar to those by PCA. The first component is again related to the average element concentration, and the second and third components provide qualitative information on the elements which more clearly influence each sample group. However, there are two differences compared to PCA which should be highlighted. Both of them probably arise from the non-perceptive orthogonality of the components. First, the differences between *GA* and *AR* (and in a less extent, between *GO* and *UD*) become directly evident from observation of the scores on the C1-C2 space, making it unnecessary to investigate the C1-C3 plot. Second, the loading plots indicate that Mn is related to Co and Ni, rather than to Al and Mg (as suggested by PCA).

a) C1 vs. C2 and C3



b) C1 vs. Sample plot

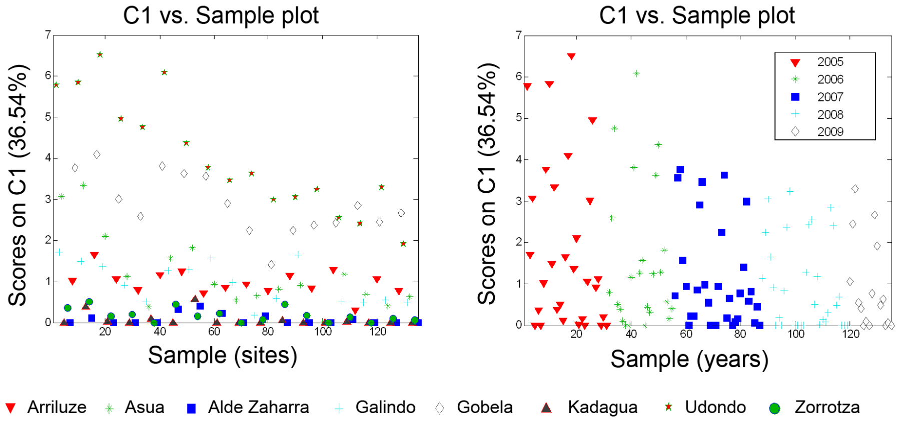


Figure 4. MCR results: scores and loadings plots on C1-C2, and C1-C3 (a); Plot of the scores on C1 together with colour grouping of samples according to sampling site and sampling year (b).

This is in good agreement with the anthropogenic origin of Mn in the estuary of the *Nerbioi-Ibaizabal* River.

The decrease in average element concentration with time observed in PCA is also noticeable in MCR, being even a bit more marked in the plot of the scores on C1 (Figure 4b).

4.2. Three-way models

Before starting with the analysis of the results obtained by Tucker3 and PARAFAC, it is to be highlighted that a general advantage of three-way models compared to two-way models consists on the fact that the arrangement of data in Tucker3 and PARAFAC allows us interpretation of the results in terms of i) sampling sites (that is, a global loading value on each component is calculated for each sampling site, including all the sediments collected at that sampling site along sampling campaigns) and ii) sampling campaigns (that is, a global loading value on each component is calculated for each sampling campaign, including all the sediments collected during that sampling campaign at the different sampling points). This is important because the graphs are highly simplified and so it is the interpretation of the results arising from them.

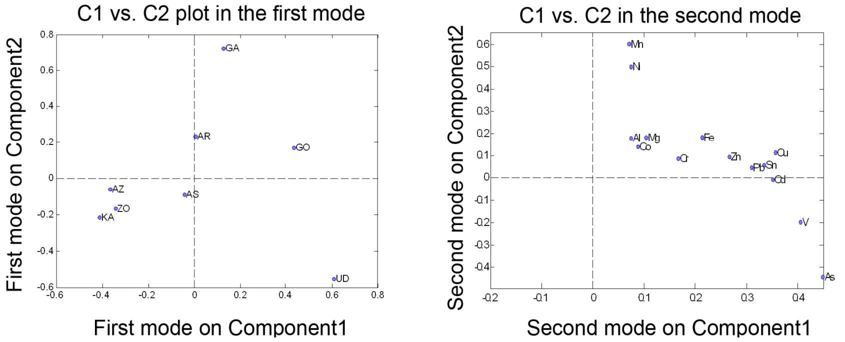
4.2.1. Tucker3

For this particular dataset it was initially found that a (Mas et al., 2010) Tucker3 model explained 74% of the variance, which was deemed sufficient. By scrutinizing the core array of the [3,3,3] model, it was decided that only one component was required in the third mode. Hence a [3,3,1] Tucker model was used to fit data, explaining 70% of the total variance.

The results obtained with this model are summarized in the Figure 5. As the third mode was explained with an unique component, it was not possible to plot loadings of this mode on bidimensional spaces. Similarly to PCA and MCR, the first component sorts sampling sites according to their overall trace element content, so the main source of variation in the dataset is again the different average element content of the sites.

Like in the case of bi-linear methods, the second and third components help us grouping sampling sites (first mode) and element concentrations (second mode), and connecting both modes. The grouping pattern arising from Tucker3 is similar to that obtained by PCA and MCR. Tucker3 emphasizes, however, the importance of Ni in the *GA* sampling point, and As in the *UD* sampling point.

a) C1 vs. C2 in the first and the second modes



b) C1 vs. C3 in the first and the second modes

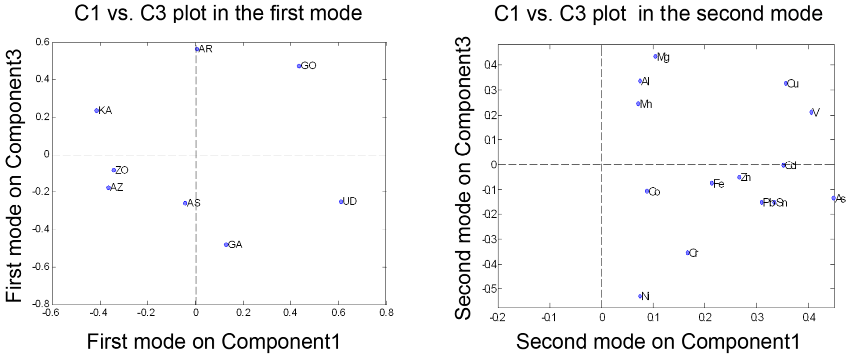
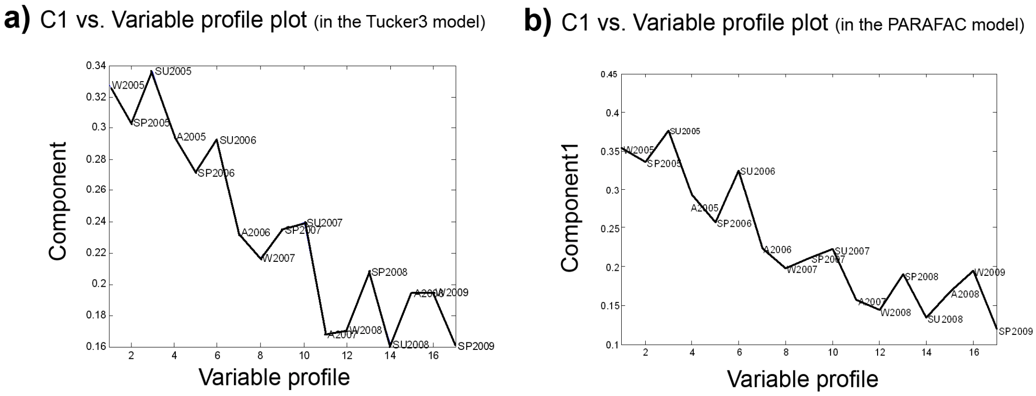


Figure 5. Tucker3 results: loadings plots on C1-C2 (a), and C1-C3 for the first and second modes (b).

Tucker3 also confirms the similarity of Al and Mg (not Mn), and the influence of these two elements (not Mn) in the *AR* sampling point.

The fact that there is only one component in the third mode implies that the variations observed and reflected in the Tucker3 model by all the components in the first and the second modes have the same time profile. Considering that the third mode includes the loadings related to the sampling campaigns, the decreasing profile observed with time (Figure 7a) was the same for the three components of the first and the second modes. Tucker3 model shows in a very clear way that the metal content in the sediments from the estuary is undoubtedly decreasing with time, a fact that was already pointed out by PCA and MCR, but not so evidently. In addition, Figure 7a also suggests a possible seasonal trend in data, with peaks of concentration approximately in summer time.



a) C1 vs. Variable profile plot (in the Tucker3 model)

b) C1 vs. Variable profile plot (in the PARAFAC model)

Figure 7. Distribution of the loadings on the first component for the third mode in the case of the Tucker3 model (a) and the PARAFAC model (b); seasons are indicated with acronyms: W; winter, SP: spring, SU: summer, AU: autumn.

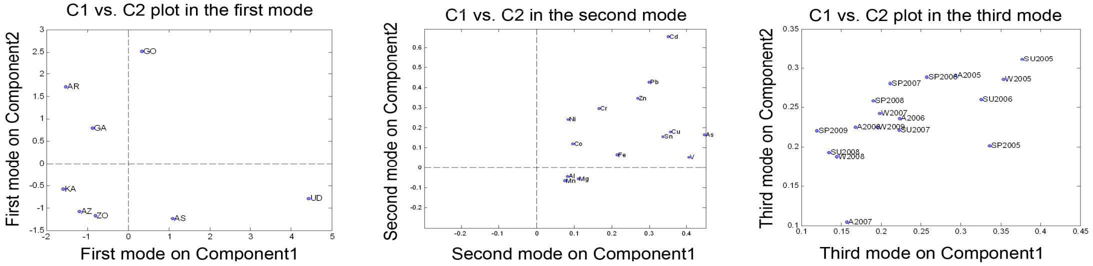
4.2.2. PARAFAC

The PARAFAC model with three components was chosen as the best one, with a 74% of explained variance and a 94% of core consistency, which measures the ‘appropriateness’ of the PARAFAC model (Álvarez et al., 2008; Bro and Kiers, 2003). Addition of a fourth component to the model results in a dramatic loose of core consistency (24%), with only a modest improvement in explained variance (82%).

The first PARAFAC component (accounting for 57% of the explained variance) also sorts the samples by their overall content in all the elements (Figure 6). The apparent clustering observed, though, is slightly different from that obtained by PCA, MCR and Tucker3: *KA*, *AZ, ZO, AR* and *GA* present negative loadings on the first component, *GO* and *AS* values close to 1, while *UD* significantly differs with a loading over 4. Grouping of sample sites provided by PARAFAC is similar to that obtained by the other methods. In this case, however, the specific characteristics of the *GA* sampling site, specifically influenced by Ni, are highlighted, and a more clear relation between the *GO* and *UD* sampling sites can be observed.

Figure 6. PARAFAC results. loadings plots on C1-C2 (a); and C1-C3 for the first, second and third modes (b).

a) C1 vs. C2 in the different modes



b) C1 vs. C3 in the different modes

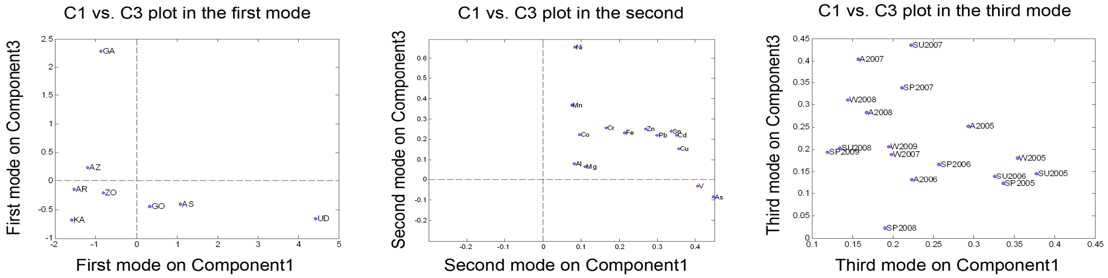


Table 2. General comparison of the obtained results by the different models. *AS*, Asua; *AR*, Arriluze; *AZ*, Alde Zaharra; *GA*, Galindo; *GO*, Gobela; *KA*, Kadagua; *UD*, Udondo; *ZO*, Zorrotza.

	Best model		First component			Second component			Third component		
	Var. (%)*	Comp.*	Var. (%)*	Observations		Var. (%)*	Observations		Var. (%)*	Observations	
				Sites	Variables **		Sites	Variables		Sites	Variables
PCA	74	3	49	Positive value: *AR, GO, UD, GA* Negative value: *AS, KA, ZO, AZ*	All metals	15	Positive value: *AR, GA* Negative value: *AS, GO, UD*	Al, Mg, Co, Ni, Cr, Mn, Fe (Positive)	10	Positive value: *AR, KA, GO* Negative value: *ZO, AZ, AS, UD*	Al, Mg, Mn V, Cu, Fe (Positive)
MCR-ALS	75	3	37	High value: *AR,GO, UD, GA* Low value: *KA, ZO, AZ*	All metals	20	High value: *GA, GO* *Lo*w value: *KA, ZO, AZ, UD, AS, AR*	Mn, Ni, Co, Cr, Fe (Highest values)	19	Positive value: *AR* Low value: *AZ, AS, ZO, GA, UD, GO, AS*	Al, Mg (Highest values)
Tucker3	70	[3 3 1]***	--	Positive value: *GO, UD, GA* Negative value: *KA, ZO, AZ, AS*	All metals	--	Positive value: *GA, AR, GO* Negative value: *KA, AZ, ZO, AS, UD*	As, V (Negative)	--	Positive value: *AR, KA, GO* Negative value: *AZ, AS, GA, AZ, ZO, UD*	Al, Mg, Mn, Cu , V (Positive)
PARAFAC	74	3	57	Positive value: *GO, UD, AS* Negative value: *AR, KA, GA, ZO, AZ*	All metals	30	Positive value: *GA, AR, GO* Negative value: *KA, AZ, ZO, AS, UD*	Al, Mg, Mn (Negative)	13	Positive value: *GA, AZ* Negative value: *AR, ZO, KA, GO, AS, UD*	V, As (Negative)

* Var.(%) accounts for percentage of explained variance for the chosen number of components (Comp.). The column of components has to be understood as PCs in PCA model and Components in MCR, PARAFAC and Tucker3 models.
** Variables with positive value in the loading plot indicated.
*** Tucker3 model with 3 components in the first and the second mode and 1 component in the third mode.

As noticed in the Tucker3 model, a decreasing trend of the loadings on the first component of the third mode can be also distinguished in the PARAFAC results. It suggests again that a cleaning process could be happening in the estuary (Figure 7b). The same possible seasonal trend observed with Tucker3 is also noticeable in Figure 7b.

5. Conclusions

The analysis of the results obtained allows us to draw some conclusions in two different directions. The first one concerns environmental considerations on the particular case investigated here. The second one refers to some issues of general application when any environmental dataset is analysed by the multivariate methods considered in this work.

Table 2 summarises the most outstanding features of the models obtained to explain the variability of the investigated dataset and, at the same time, highlights the differences among them. All the tested methods produced models with similar percentages of explained variance (around 75%), which could be considered enough taking into account the high variability that environmental datasets usually contain (Peré-Trepat et al., 2007; Smoliński et al., 2008).

It should be noted that, whatever the method is, the first (principal) component always sorts samples according to their average element content. The second and third components are useful to group sampling sites and/or element concentrations with similar characteristics, and to connect sampling sites with element concentrations. The samples from *GO* and *UD* systematically present the highest loading values on the first component. PARAFAC also includes *AS* among the most polluted sampling sites. All the methods identify *AZ, KA* and *ZO* as the sampling points with the lowest average element concentration. All the methods agree in giving similar metallic characteristics to the *AZ, KA* and *ZO* sampling points, and in highlighting the differences between *AR* and *GA*, and *GO* and *UD*. A decreasing pattern with time in the average element content of some of the sampling sites (*AS, GO* and *UD*) was clearly distinguished by PCA and MCR. The three-way models confirmed this and both PARAFAC and Tucker3 were also able to observe a possible seasonal pattern, with maximum values of metal content in summer time.

Under a practitioner perspective, the application of the three-way models may be more difficult than the use of the two-way models. This is both due to practical and theoretical aspects. On the one hand, the creation of the augmented data commonly turned out more difficult in the case of the three-way methods, due to the complexity that entailed the arrangement of the samples and the variables in a three-dimensional abstract structure. Moreover, there are few available software packages that include multi-way methods and, in addition, determining the appropriate model to use (the number of components) can be more cumbersome. In the case of two-way models, even if additional non-useful components are included, the interpretation of the most significant components does not significantly change. Application of Tucker3 may result even more complex, because the core array has to be always considered. This gives information about important interactions between modes, so depending on its values the relation among the modes will change. In the particular case investigated in this work, the core array values were all positive, so there was a direct relation between the components of the modes. Nevertheless, when the core array indicates an indirect relation between the modes (one or some of the core array values are negative), the interpretation of the model becomes more complicate. However, the use of three-way methods involves several advantages: i) representation of the results in comprehensive plots is highly simplified, and so it is the extraction of conclusions applicable to the whole mode (in the specific case studied here, sampling site or sampling campaign), and ii) the identification of general trends in data with variables which have not been directly measured (time, position in the estuary,…) is simplified. It should also be noted that the non-perceptive orthogonality of the components in MCR (regarding to two-way methods) and in Tucker3 (according to three-way methods) may give rise to slightly different conclusions compared to the PCA and PARAFAC respectively. The goodness of those conclusions highly depends on the dataset analysed, and should be established by the experienced researcher based of her/his previous knowledge of the system under study.

To finish, although the results observed after the use of the different chemometric methods varied slightly, the main conclusions extracted from them were similar and they could be considered complementary. Taking into account the pro's and con's of each method, the use of a two-way chemometric method may be considered enough in the environmental risk assessment of a specific area. Nonetheless, and generally speaking, if the aim of the work is the exhaustive study of an area that is unknown, a combined application of all the methods might be advisable.

Acknowledgements

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3. Atala

Analisi-metodo alternatiboen garapena

Analisi-metodo berdeen garapena: teknika ez-suntsikorren eta kimiometrikoen erabilera

Tradizionalki gizarteak Kimikaren inguruan izan duen pertzepzioa ez da batere positiboa izan. Industria-Iraultzak industria astunaren neurririk gabeko garapen bortitza ekarri zuen. Honek gogorki kutsatu eta eraldatu zituen ur naturalak, lurrak eta atmosfera, jarduera industrialetan sorturiko hondakin guztiak zuzenean eta inolako tratamendurik gabe isurtzen baitziren ingurumenera. Garai hartan, beraz, oso zaila zen Kimika eta kontaminazioa estuki ez lotzea.

Gaur egun Kimika ulertzeko modua errotik aldatu da, eta honek zeukan kutsu negatiboa arinduz doa. Kimika eta kutsadura lotzeko joera oraindik nabarmena bada ere, ingurumena hobetzeko eta babesteko erreminta bat bezala ere kontsideratzen hasi da Kimika.

Azken bi hamarkadetan *garapen jasangarri*, *ingurumenaren babesa* edota *ekologia* bezalako terminoak gero eta maizago erabiltzen ari dira. 1990. urtean Paul Anastas-ek eta John Warner-ek korronte honekin bat egiten duen *kimika berdea*ren kontzeptua proposatu zuten [1]. *Kimika berdea* ikerkuntza kimikorako eta ingeniaritzarako filosofia berri bat moduan definitu zuten, produktu kimikoen diseinuan, sintesian eta aplikazioan arriskutsuak diren substantzien eraketa edo erabilera eliminatu edo behintzat gutxituko zituen bide bat bezala. Hau lortzeko ingurumenarekiko begirunea (“environmentally friendly”) erakusten duten 12 printzipio definitu zituzten [1]: i) prebentzioa: aukeran hobe da hondakinen eraketa eragoztea, hauen garbiketa edota tratamendua burutzea baino; ii) ekonomia atomikoa garatzea: prozesuan erabilitako materialen transformazioa azken produktuan ahalik eta handien izateko helburuarekin diseinatu behar dira sintesirako metodoak; iii) arriskutsuak diren sintesi kimikoak gutxitzea; iv) produktu seguruak diseinatzea: toxizitate baxuko produktu kimikoak eratu behar dira, beti ere beraien funtzioaren eraginkortasuna bermatuz; v) substantzia laguntzaileen (disolbatzaileen, banaketarako erreaktiboen eta abarren) erabilera mugatzea; vi) etekin energetikoa optimizatzea, posiblea den neurrian, sintesirako metodoak inguruko tenperaturan eta presioan garatuz; vii) material berriztagarriak erabiltzea; viii) deribatizazio-prozesuak, ahal den neurrian, sahiestea; ix) erreakzio katalitikoak bultzatzea; x) beraien funtzioa bete ostean, denboran iraunkortzen ez diren hondakinak ematen dituzten produktu kimikoak diseinatzea; xi) unean uneko monitorizazioa ahalbideratzen duten metodologia analitikoak garatzea, substantzia arriskutsuen eraketa antzeman ahal izateko;

eta azkenik xii) kimika segurua zabaltzea, istripuak gertatzeko posibilitateak gutxituz. *Kimika berdea*k, orokorki, merkatuaren, ingurumenaren eta gizartearen interesak uztartzea eskatzen du.

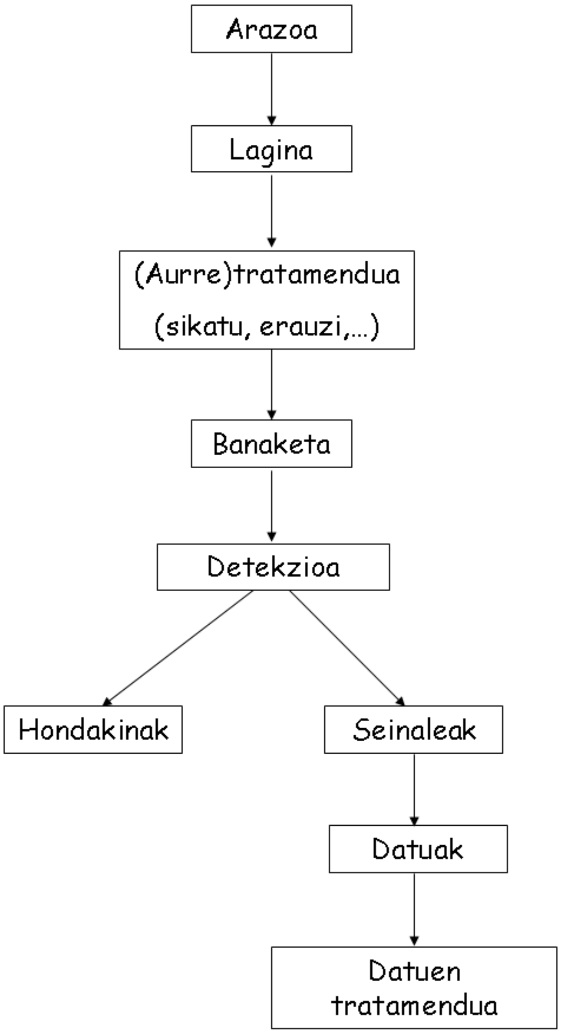
*Kimika berdea*ren kontzeptuak Kimika Analitikoaren arlora ere ekarri dira. *Kimika Analitiko berdea*k, besteak beste, arriskutsuak diren erreaktiboen erabilera saihestea, edo neurri handi batean gutxitzea, eta energia-kontsumoa eta hondakinak murriztea eskatzen ditu. Baina *Kimika Analitiko berdea* ez da analisi-metodoaren urratsetara mugatzen, ingurugiroaren babesari egokituta dagoen prozedura analitiko osoa proposatzen du, non laginketa edo laginaren aurretratamendurako urratsak eliminatu edo behintzat minimizatu behar diren, eta eraturiko hondakinen garbiketa ere urrats garrantzitsua den [2].

*Kimika Analitiko berdea*ren garapenerako funtsezkoa da, beraz, ingurumenarekiko begirunea erakusten duten teknika analitiko berrien garapena edo jadanik erabiltzen diren metodoen moldaketa. Xede honekin lan ugari burutu dira, adibidez, laginaren aurretratamendua arintzeko edo saihesteko arloan, kontrol urruneko sentsoreen garapenean eta teknika ez-suntsikorren aplikazioan [3, 4].

Kimika tradizionalean helburu kuantitatiboak dituen edozein prozedura analitikok 1. Irudian ikus daitezkeen urratsak biltzen ditu ia salbuespenik gabe. Analizatu eta kuantifikatu nahi denaren arabera, urratsok era espezifiko batean burutzen dira, baina pausu guztietan, osasunerako kaltegarriak diren hondakinak eratzeaz aparte, energia-kontsumoa nabaria izaten da.

Laginketak, normalean, ez dio ingurumenari arrisku handirik ekartzen, beti ere materialaren garbiketarako beharrezkoak diren produktuak behar bezala aukeratzen eta maneiatzen badira. Azken urteotan, gainera, laginketa tradizionalak (laginketa aktiboak) eskatzen dituen denbora eta kostu ekonomikoak minimizatzeko, gero eta gehiago erabiltzen ari da laginketa pasiboa. Laginketa pasiboak eskatzen duen aurretratamendua oso sinplea da [5].

Edozein lagin solidok zein likidok (aurre)tratamendu zehatza eskatzen du, lagin solidoetan luzeagoa, garestiagoa, eta ingurumenarekiko arriskutsuagoa izaten dena. Hurbilketa klasikoan analitoak fase likido batera erauzi behar izaten dira. Prozesua luzea izaten da, eta lagina kutsatzeko edo analitoak eraldatzeko arriskua, berriz, handia. Konposatu ez-organikoen kasuan erauzketa sekuentzialak [6], erauzketa *pseudo*-osoak (gutxienez bioeskuragarria den analitoaren frakzioa berreskuratzen da) [7] eta erauzketa osoak [8] erabiltzen dira. Kasu guztietan azido mineral ugari erabiltzen dira. Gainera, prozesuak luzeak izaten dira, denbora eta energia asko galduz. Mikrohuinezko eta ultrasoinuzko energia eta presiopeko teknikak erabiltzea saiatu da erauzketa-denbora murrizteko, baina energiaren kontsumoa ez da hobetu [9].



1. Irudia. Prozedura analitikoaren urratsak hurbilketa klasikoan.

Konposatu ez-organikoen banaketan teknika kromatografikoak eta elektroforetikoak dira erabilienak. Detekzioan, berriz, masa-espektrometria eta teknika atomikoak dira ohikoenak, erakusten duten selektibotasun altuagatik eta aldiberekotasunagatik [10-12]. Banaketa eta detekzioa, alabaina, eta instrumentazioaren arloan gertatutako garapen ikaragarriaren ondorioz, ez dira gaur egun *Kimika berde*aren ikuspuntutik urrats kritikoak.

Antzeman daitekeenez, ingurumeneko lagin solidoen analisirako betiko metodoek garestiak, luzeak, eta ingurugiroarekiko zein gizakiarekiko arriskutsuak diren prozesu ugari biltzen dituzte. Hori hobetzeko asmoz, eta *Kimika Analitiko berde*aren printzipioak aurrera eraman nahian, azken urteotan sakonki aztertu den arlo bat teknika espektroskopiko ez-suntsikorren erabilpenarena izan da [3]. Hauen aplikazioak, era guztietako laginetara hedatu den arren, lagin solidoen analisian izan duen arrakasta nabarmena da [13-17]. Teknika ez-suntsikor moduan ezagutzen dira laginak bere horretan jarraitzen duelako analisia egin ondoren. Gainera, kasu gehienetan, ez du laginaren tratamendurik (erauzketarik edo liseriketarik) eskatzen. Hau da, hain justu, *Kimika berde*aren ikuspuntutik, haien ekarpenik garrantzitsuena. Gainera, laginketa-urratsa sahiestea ere ahalbidetzen dute, *in-situ* analisiak buru baitaitezke ekipo eramangarriak erabiliz [14, 18]. Teknika ez-suntsikorren beste abantaila bat neurketa bakar batean osagai ezberdinen aldi bereko analisia ahalbideratzea da, erreaktiboak eta denbora aurreztuz.

Azken hamarkadan, gehien garatu diren teknika espektroskopiko ez-suntsikorren artean teknika bibrazionalak (infragorri ertaineko espektroskopia, *Mid Infra-Red, MIR*, infragorri-hurbila, *Near Infra-Red, NIR*, eta Raman espektroskopia) eta X izpiko fluoreszentzia, *X-Ray Fluorescence, XRF*, daude. Edozein teknika espektroskopikotan gertatzen den moduan, erradiazio elektromagnetikoaren eta materiaren arteko elkarakzioetan oinarritzen dira, eta erabilitako uhin-luzeraren arabera lortzen den informazioa desberdina da.

Infragorriko espektroskopiaren kasuan, aztergai den molekula bere bibrazio-mugimenduaren pareko energia duen argiarekin irradiatuz gero, molekularen momentu dipolarra alda daiteke, espektroaren tarte infragorrian seinalea emanez. Xurgapen-espektroa aztertuz, molekularen informazio koantitatiboa eta kualitatiboa lor daiteke. Azken urteotan, infragorriko tekniken artean NIR espektroskopia (780-2500 nm; 12800-4000 cm^{-1}) da nagusitu dena. Laborategi- zein industria-mailan erabiltzen da askotan, batez ere elikagaien, lagin farmazeutikoen, pieza arkeologikoen eta ingurumeneko laginen analisi molekularrean [14, 19-23]. NIR teknikaren espektroen interpretazioa MIR edo FIR, *Far Infra-Red*, tekniken espektroena baino zailagoa da, C-H, N-H eta O-H bezalako talde funtzionalen loturei dagozkien seinaleen gainjartzea sarri gertatzen delako. NIR espektroskopiaren modalidaderik arruntena xurgapena izan arren, azken aldian islatutako argian oinarrituriko zenbait teknika garatu dira, DRIFT, *Diffuse Reflectance Infrarred Fourier Transform*, eta ATR, *Attenuated Total Reflectance*, besteak beste [24, 25].

X izpien erradiazioak materian duen eragina ezberdina da. X izpiek, askoz energia handiagoakoak izanik, atomoen barne-elektroiak erauztea lortzen dute, barne-geruzetan hutsune negatiboak utziz. Hutsuneak kanpoagoko elektroiek betezen dituzte, erlaxazio-prozesuan elementu bakoitzaren kasuan bereizgarria den energia igorriz fluoreszentzia moduan. Beraz, XRF-k, teknika bibrazionalek ez bezala, informazio elementala ematen du. X izpien bidezko espektroskopia medikuntza arloko eta ingurumenari buruzko ikerketa ugarietan erabili da [26-29]. Azpimarratzekoa da teknika bibrazionalen eta elementalen arteko konbinazioak saiatzea asko zabaldu dela azken aldian. Honen adibide gisa, SEM-Raman-EDX, *Scanning Electron Microscopy coupled with Energy Dispersive X-ray and Raman spectrometry detection*, dugu [30, 31]. Teknika akoplatu honen bitartez posiblea da aztergai den laginari buruzko informazio elementala (XRF-k ematen duena) eta molekularra (Raman-ak errazten duena) uztartzea.

Beste aldetik, kimiometria delako arloa garrantzia berezia bereganatzen ari da azken hamarkadetan *Kimika berdea*ren kontzeptuarekin bat datozen metodo analitikoen garapenean. Kimiometria kontzeptua lehen erabili zuena Svante Wold suediarra izan zen, XX. mendearen 70. hamarkadaren hasieran [32]. Bruce R. Kowalskirekin elkarlanean, International Chemometrics Society sortu zuen 1974an. 80. hamarkadan jaio ziren kimiometriari buruzko lehenengo bi aldizkari espezializatuak: *Chemometrics and Intelligent Laboratory Systems* (1986) eta *Journal of Chemometrics* (1987). Gaur egun honelaxe defini daiteke kimiometria: metodo matematikoak eta estatistikoak erabilitzen dituen kimikaren arloa, i) datuak ebaluatzeko eta interpretatzeko, ii) prozesuak eta esperimentuak modelatzeko eta optimizatzeko, eta iii) datu esperimentaletatik abiatuta ahalik eta informazio kimiko gehien lortzeko [33].

Teknika kimiometrikoak asko diren arren, ingurumeneko kimika analitikoan ondokoak dira nagusiki erabiltzen direnak :

- *Denborarekiko serien analisirako teknikak* (Time series analysis): ingurumeneko kimika analitikoan, eta espezifikoki monitorizazio-lanetan, askotan erabiltzen dira denborarekiko serieen analisirako teknikak, besteak beste, analitoek denborarekin erakutsi ditzaketen balizko joera sistematikoak detektatzeko. Kronologikoki ordenaturiko datu-bildumen analisiaren helburu nagusiak lau taldetan bana daitezke: iragarpena, prozesuen kontrola, prozesuen simulazioa y teoria fisiko eta biologiko berrien proposamena. Multzo honetan erabiltzen diren metodoen artean seinaleen leunketa eta iragazpena, erregresio-teknikak, korrelazio-teknikak eta korrelazio gurutzatua, autoerregresioa eta ARIMA, *AutoRegressive Integrated Moving Average*, delako modelatzea aipa daitezke [33, 34].
- *Seinale analitikoen tratamendurako teknikak:* seinale kromatografikoak eta espektralak askotan erabiltzen dira kimika analitikoan. Espektro edo kromatograma batetik kalitatezko informazio erabilgarria eta adierazgarria

lortzeko seinaleen aurretratamendua beharrezkoa izaten da. Zereginik garrantzitsuenak arlo honetan oin-lerroaren zuzenketa, seinaleen lerrokatzea eta datuen normalizazioa dira. Oin-lerroaren distortsioak eliminatzeko *Offset correction* eta *Detrend correction* delako teknikak eta seinalearen deribatizazioa erabiltzen dira. Seinaleen desplazamendu lineala *Coshift* izeneko algoritmoaren bidez zuzendu daiteke. Desplazamendu ez-linealak edo ez-sistematikoak zuzentzeko, berriz, DTW, *Dynamic Time Warping*, eta COW, *Correlation Optimized Warping*, teknikak erabiltzen dira. Espektroen kalitatea normalizazio-tekniken bidez hobetu daiteke. Hauen helburua datuen soberazko informazioa eliminatzea da, informazio esanguratsua nabarmenduz. Honetarako espektroak zentratu, eskalatu, leundu eta eraldatu egiten dira, beste aukera batzuen artean [35-37].

➢ *Aldagai anitzeko metodoak:* aldagai anitzeko datu-bildumen aldi bereko analisian erabiltzen diren metodo estatistikoen multzoa da. Lau talde nagusitan bana daitezke: i) datuen errepresentaziorako teknikak, ii) multzoen identifikaziorako teknikak, iii) sailkapenerako teknikak eta iv) erregresiorako teknikak.

- Datuen errepresentaziorako teknikak datu-matrizearen ezaugarri nagusiak nabarmentzeko, laburbiltzeko edo sinplifikatzeko erabiltzen diren teknika grafikoak dira, gehienetan kalkulu estatistiko sinpleetan oinarritzen direnak. Hauen helburua datuen egitura orokorra era azkar batean irudikatzea da, oinarrizko kalkulu estatistikoak erabiliz. Datuen bilduma handien tratamenduan maiz aplikatzen dira laginei buruzko lehen ideia orokor bat eskuratzeko. Erabilitako metodoen artean normalizazioa eta eskalatua, korrelazio-analisia, loturen bidezko teknikak (*nesting techniques*), izarren bidezko grafikoak (*star plots*), irudien bidezko errepresentazioak (*pictorial representation*), Box-Whisker delako diagramak eta histogramak daude [33, 38].
- Multzoen identifikaziorako tekniken helburua antzeko ezaugarriak dituzten objektuen (laginen) edo aldagaien (esperimentalki neurtutako parametroen) multzoak identifikatzea da. Modu horretan datu-matrizearen egitura orokorraren azterketa egitea ahalbideratzen dute. Datuak nola antolatzen diren arabera, bi edo hiru dimentsiotako teknikak bereizi daitezke. Metodo bi-dimentsionalen artean CA, *Cluster Analysis*, PCA, *Principal Component Analysis*, FA, *Factor Analysis*, MCR, *Multivariate Curve Resolution*, MCR-ALS, *Multivariate Curve Resolution Alternating Least Squares*, TTFA, *Target Transformation Factor Analysis*, eta PMF, *Positive Matrix Factorization*, dira gehien aplikatu direnak [39-45]. Hiru-dimentsionalen artean, berriz,

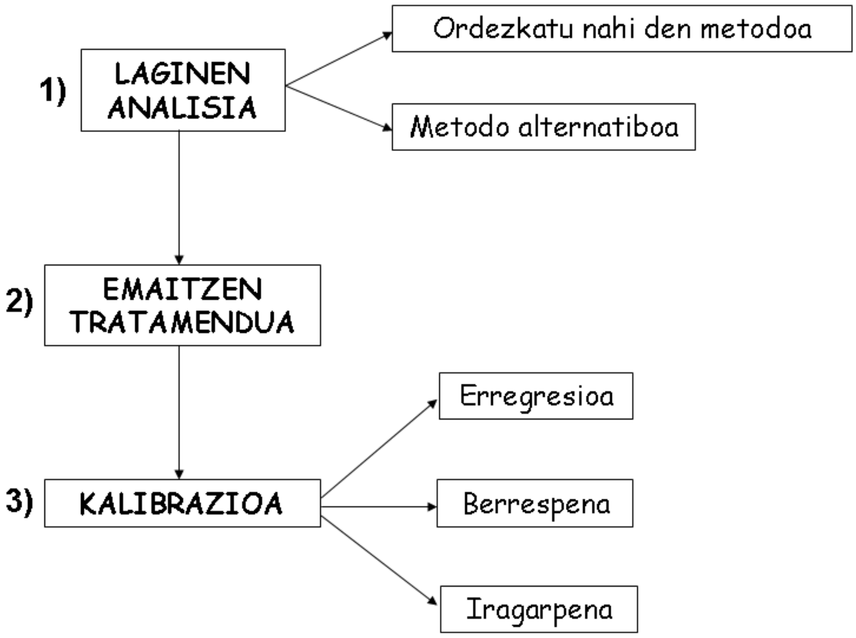
STATIS, *Structuration des Tableaux A Trois Indices de la Statistique*, PARAFAC, *Parallel Factor Analysis*, PARAFAC 2 eta Tucker3 [46-52] daude.

- Sailkapenerako teknikak datuen bildumetan laginak beraien ezaugarrien arabera sailkatzeko erabiltzen dira. Bi taldetan bana daitezke: datu-matrizean dagoen informazioa besterik erabiltzen ez dutenak (ikuskatugabeko teknikak, *non-supervised techniques*), alde batetik, eta objektuen (laginen) informazio gehigarria erabiltzen dutenak (ikustatutako teknikak, *supervised techniques*). Lehen multzoan lehen aipatutako multzoen identifikaziorako teknika gehienak sar daitezke, besteak beste, CA (bere modalidade desberdinetan, hots, *single linkage method* eta *k-means method,* besteak beste) eta PCA. Bigarren multzoko tekniketan laginen artean diferentziaren bat ezartzen duen ezaugarren bati buruzko informazioa erabiltzen da, sailkatzerako eredu bat proposatzea ahalbideratzen duena. Lagin berriak multzo batean edo bestean sailkatu daitezke gero ereduaren arabera. Teknika hauen artean LDA, *Linear Discriminant Analysis*, k-NN, *k-Nearest Neighbour*, SIMCA, *Soft Independent Modeling of Class Analogy*, eta ANN, *Artificial Neural Networks*, aipa daitezke [53-57].

- Azkenik erregresiorako teknikak aldagai anitzeko kalibrazioan erabiltzen dira, bi matrizeen arteko erlazioa eredu matematiko baten bidez finkatzeko. Matrize bakoitzak objektuen (laginen) multzo berdinaren informazio desberdina biltzen du. Lehenak ordezkatu nahi den metodo analitiko baten bidez lortutako informazioa; bigarrenak, berriz, metodo alternatibo baten bidez lortutakoa. Ingurumeneko kimika analitikoan metodo bat ordezkatu nahi izateko arrazoiak anitzak izan daitezke, besteak beste, metodoa garestia izatea, kimika berdearen kontzepturen batekin bateragarria ez izatea edota prozedura esperimentala luzea eta nekosoa izatea [58]. Aldagai anitzeko erregresiorako tekniken artean PLS, *Partial Least Squares*, da diferentziaz nagusitu dena [58] baina, hala ere, beste metodo lineal batzuk ere garatu eta aplikatu izan dira: GAPLS, *Genetic Algorithms Partial Least Squares*, MLR, *Multiple Linear Regression*, FA/MLR, *Multiple Linear Regression with Factor Analysis*, RRR, *Reduced Rank Regression* eta PCR, *Principal Component Regression*, beste batzuen artean. Hauetaz aparte, linealak ez diren metodoak ere aipa daitezke, hots, ANNs, *Artificial Neural Networks*, eta SVM, *Support Vector Machine*, esate baterako [59-65].

Tesi honen hurrengo ataletan aldagai anitzeko kalibrazioa erabiliko da sedimentuetan metalen kontzentrazioa neurtzeko metodo analitiko alternatiboak proposatzeko.

Kalibrazio-prozesuetan 1. urratsa patroi bezala erabiliko diren laginei buruzko informazioa eskuratzea da (2. Irudia). Alde batetik, ordezkatu nahi den metodoak ematen duena (askotan, analito desberdinen kontzentrazioa), eta bestetik, metodo alternatiboak ematen duena (teknika espektroskopikoetan, adibidez, espektroa).



2. Irudia. Aldagai anitzeko kalibrazioen eskema orokorra.

2. urratsan, lortutako informazioaren aurretratamendua burutu behar da. Neurtutako seinalea espektrala bada, informazio erabilgarria eta adierazgarria eskuratu behar da, aurretik aipatu diren seinale analitikoen tratamendurako metodoen bitartez, hots, oin-lerroaren zuzenketa, seinalearen leuntzea, normalizazioa, eta abar. Batzuetan, informazio gehien ematen duen espektroaren edo kromatogramaren zatia aukeratzea ere komenigarria izaten da. Askotan informaziorik ematen ez duen espektroaren zatiak erabiltzeak eredua hobetu ez ezik okertu ere egin dezake, batez ere bere sendotasunari dagokionez.

3. urratsa kalibrazioa bera da. Urrats honetan hiru etapa desberdintzen dira, hots, erregresioa, berrespena eta iragarpena. Ezer baino lehen, laginak bi azpimultzotan banatu behar dira, erregresiorako eta berrespenerako multzo delakoak. Azpimultzo bakoitzak esperimentalki neurtu diren parametroetan

laginek erakausten duten aldakortasun osoa islatu behar du. Zeregin honetan oso lagungarria izaten da datu-matrizeen multzoen identifikaziorako teknikaren bat (PCA gehinetan) erabiltzea, eta lortutako emaitzak kontutan hartzea laginen banaketa egiteko orduan. Analisi hau erabakiorra izan daiteke, erregresiorako eta berrespenerako multzo errepresentatiboak egitea ahalbidetzen duelako. Erregresiorako multzoa erregresioan, hots, eredu matematikoaren eraketan, erabiltzen da. Laginen %75 azpimultzo honetan egotea gomendatzen da [58]. Aldagai anitzeko erregresioan gehien aplikatu den teknika PLS, *Partial Least Squares*, delakoa izan da, baina MLR, *Multiple Linear Regression* edo PCR, *Principal Component Regression*, ere erabili izan dira [58]. Berrespenerako multzoa, berriz, eredua berresteko erabiltzen da. Laginen %25 hartzen du gutxigorabehera. Berrespen prozesuan erregresioan eratutako ereduaren kalitatea neurtzen da. Eredua erabiltzen da berrespenerako multzoko lagin bakoitzari dagozkion balio teorikoak estimatzeko, eta hauek esperimentalekin konparatzen dira, errorea kalkulatuz.

Erregresiorako eta berrespenerako eskuartean ditugun laginen kopurua laburregia den kasuetan, erregresioa eta berrespena laginen multzo osoa erabiliz egin daitezke. Berrespena-prozesuaren emaitzak ez dira hain fidagarriak, baina erregresioa egiteko laginen kopurua altuagoa da, ereduaren kalitatearen gainean eragin positiboa duena. Kasu honetan, ereduaren berrespena egiteko bi teknika erabil daitezke, hots, *cross validation* (balidazio gurutzatua) eta *leverage validation* (pisuen araberako berrespena) delakoak [58].

Kalibrazioaren azken etapa iragarpena da. Etapa honetan ereduaren eraginkortasuna frogatzen da erregresiorako eta berrespenerako multzoekiko guztiz independentea den beste laginen multzo batekin. Iragarpenerako multzoko laginek bete behar duten baldintza bakarra esperimentalki neurtutako aldagaien aldakortasun-mugen barruan egotea da. Modu honetan ziurtatzen da iragarpena interpolazio-prozesu baten ondorioa dela, ez estrapolazio-prozesu baten emaitza.

Sarrera honetan deskribatutakoa kontutan hartuz, eta kimika analitiko berdearen kontzeptuei zein Europako Uraren Zuzentarauaren gomendioei egokitzeko bidean, lan honen hurrengo bi ataletan metalen kontzentrazioak sedimentuetan determinatzeko bi metodo alternatiboen garapena eta optimizazioa deskribatzen dira. Hurbilketa klasikoan sedimentuen erauzketa azidoa burutu behar da analisia egin baino lehen. Proposatutako metodoek urrats hori saihesten dute, analisia sedimentu lehorrean zuzenean eginez. Lehen atalean NIR, *Near Infrared*, eta FT-IR, *Fourier Transform Mid Infrared*, teknikak erabiltzen dira analisian, PLS eredu sendo bana proposatuz.

Bigarrenean, berriz, XRF, *X-Ray Fluorescence*, teknikaren bidez lortutako sedimentuen espektroak erabiltzen dira PLS eredu baten bidez laginetan dauden metalen kontzentrazioak iragartzeko.

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Use of reflectance infrared spectroscopy for monitoring the metal content of the estuarine sediments of the Nerbioi-Ibaizabal River (Metropolitan Bilbao, Bay of Biscay, Basque Country)

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Use of reflectance infrared spectroscopy for monitoring the metal content of the estuarine sediments of the Nerbioi-Ibaizabal River (Metropolitan Bilbao, Bay of Biscay, Basque Country)

Abstract

Multivariate partial least-squares (PLS) calibration models have been developed for the spatial and seasonal simultaneous monitoring of 14 trace elements (Al, As, Cd, Co, Cr, Cu, Fe, Mg, Mn, Ni, Pb, Sn, V, and Zn) in sediments from 117 samples taken in the estuary of the Nerbioi-Ibaizabal River. Models were based on the chemometric treatment of diffuse reflectance near-infrared (NIR) and attenuated total reflectance (ATR) mid infrared (MIR) spectra, obtained from samples previously lyophilized and sieved with a particle size lower than 63 μm. Vibrational spectra were scanned in both, NIR and MIR regions. Developed PLS models, based on the interaction between trace elements and organic mater provide good screening tools for the prediction of trace elements concentration in sediments.

Keywords: Partial-least-squares; infrared, reflectance, estuarine sediments, metals, cluster analysis

1. Introduction

Estuaries pollution is due to anthropogenic activity that influences the physical and chemical characteristics of the system (*1*). The contamination of estuarine environments by trace metals raises special interest because metals cannot be eliminated and tend to accumulate, thus representing a serious risk to the human health (*2*). Potentially toxic elements can be involved in chemical and biological reactions as well as to interact with sediment components as minerals, humic substances, metal oxides, microorganisms, and/or ligands, depending on physical-chemical and biological conditions in the estuary. Moreover, biogeochemical processes can affect the fate and bioavailability of metals and metalloids in sediments (*3*). Due to the dynamic character of estuaries, they must be regularly monitored to evaluate seasonal and geographical chemical and physical variations of surface sediments (*4*).

For trace element determination conventional methods are based on wet digestion with hot concentrated acids followed by inductively coupled plasma (ICP) or atomic absorption spectrometry (AAS) (*5*), being in general complex, tedious, expensive, and highly time-consuming (*6*). So, for monitoring programs requiring the determination of several parameters in a high number of samples, fast and well validated methods are required. However vibrational spectroscopy provides unique tools for the direct determination of different parameters in untreated solid samples, thus offering a green analytical alternative (*7*).

Vibrational measurements have been applied to determine metals in soils and sediments (*8-16*) based on the interaction of the organic matter bands with trace elements (see Supporting Information (SI) Table S1), and it can be seen that only in two works, sediment samples were analyzed. Moreover, it must be noticed that, in our knowledge, there is only one precedent based on the use of MIR measurements to determine metals in soils (*10*). So, the aim of this work has been the development of fast, accurate and reagent free analytical methods for the simultaneous evaluation of 14 elements (Al, As, Cd, Co, Cr, Cu, Fe, Mg, Mn, Ni, Pb, Sn, V, and Zn) in estuarine sediments from the Nerbioi-Ibaizabal River (Metropolitan Bilbao, Bay of Biscay, Basque Country) using measurements in both, NIR and MIR domains, and multivariate calibration.

2. Experimental Section

2.1. Apparatus and Reagents

Two Bruker Fourier transform spectrometers were employed for spectra acquisition: (i) a near-infrared (NIR) model multipurpose analyze (MPA) equipped with an integrating sphere as measurement accessory, and (ii) a Fourier transform middle infrared (FT-IR) model Tensor 27 equipped with a KBr beamspliter, a DLaTGS detector and a three-reflection diamond/ZnSe composite DuraSampl*IR* accessory. Instrumental and measurement control, data acquisition, spectra treatment, and data manipulation were carried out using OPUS program Version 4.2 from Bruker Gmbh (Bremen, Germany).

Reference trace element concentrations used for building the prediction models were obtained by inductively coupled plasma/mass spectrometry (ICP/MS) made with an Elan 9000 ICP/MS spectrometer (Perkin-Elmer, Ontario, Canada), with Ryton cross-flow nebulizer, Scott-type double pass spray chamber and standard nickel cones. Preparation of calibrations and analysis of samples was done inside a clean room (class 100).

Table S1. State-of-the-art of previously published papers about the determination of metals in soil and sediment samples using vibrational procedures. For critical comparison, only data which belong to those metals evaluated in the present study were summarized.
(*) The number of PLS factors indicated correspond to NIR/MIR measurements.

Sample	Metals	Chemometric technique	Number of factors	Spectral range (nm)	Spectral Data	Total number of samples	Validation procedure	RPD values	Ref.
North-western Ontario (Canada) sediments	Cd, Cu, Fe, Mn, Ni, Pb, Zn	Partial Least Squares Regression (PLS)	3 for Cu, Fe, Mn, Ni, Pb and Zn 10 for Cd	1100-2500	First derivative	169	odd-numbered spectra as calibration set and even-numbered spectra as prediction set	1.74 3.35 2.64 3.66 2.96 2.45 3.80	(10)
Uruguay soils	Cu, Fe, Mg	Modified PLS (MPLS)	-----	400-2500 (Vis-NIR)	First derivative	332	Cross-Validation	-----	(11)
Tarnowskie Gory (Poland) soils	Cd, Cu, Fe, Ni, Pb, Zn	PLS	5/4 for Cd (*) 7/10 for Cu (*) 9/6 for Fe (*) 4/5 for Ni (*) 5/7 for Pb (*) 6/3 for Zn (*)	400-2500 (NIR) 2500-25000 (MIR)	Raw data	70	Cross-Validation	-----	(12)
Aznalcollar (Spain) soils	As, Cu, Pb, Zn	MPLS	-----	400-2500	First derivative	100	70 samples for calibration and 30 for an external validation	2.25 1.59 2.13 1.96	(13)
Krakowsko-Częstochowska (Poland) soils	Pb, Zn	MPLS	3 for Pb 3 for Zn	400-2500	Second derivative	74	Group Cross-validation	1.3 1.1	(14)

Table S1 (continued). Same explanation as indicatedin Table 1.

For critical comparison,

Sample	Metals	Chemometric technique	Number of factors	Spectral range (nm)	Spectral Data	Total number of samples	Validation procedure	RPD values	Ref.
Baguazhou Island (China) soils	As, Cd, Co, Cr, Cu, Ni, Pb, Zn	PLS	-----	400-2500	Raw and first derivative data	61	Cross-validation	1.90 1.23 2.18 2.50 1.74 2.35 1.49 1.45	(15)
United States soils	Cu, Fe, Mg, Mn, Zn	PCR	14 for Cu 9 for Fe 14 for Mg 12 for Mn 9 for Zn	1300-2500	First derivative	802	Cross-validation	0.92 1.66 1.78 1.79 1.07	(16)
Ria de Arousa (Spain) sediments	As, Cd, Cr, Pb, Sn	PLS	1 for As 6 for Cd 2 for Cr 3 for Pb 5 for Sn	833-2976	First derivative	81	31 samples for calibration and 50 samples for validation	1.4 1.3 1.6 1.2 1.7	(17)
Murcia (Spain) soils	As, Cd, Co, Cr, Cu, Ni, Se, Tl, Zn	PLS	1 for As 2 for Cd 4 for Co 2 for Cr 1 for Cu 5 for Ni 3 for Se 2 for Tl 2 for Zn	800-2600	First derivative	148	39 for calibration and 109 samples for validation	1.3 1.0 1.4 1.4 1.1 1.3 1.0 1.2 1.1	(18)

Nitric acid (69%, Tracepur) and hydrochloric acid (36%, Tracepur) were purchased from Merck (Darmstadt, Germany). Milli-Q (Millipore, Billerica, MA) quality water with a conductivity lower than 0.05 $\mu S \cdot cm^{-1}$ was used for samples and standards dilutions. Multielemental standard solutions of the analytes were prepared by weight in 1% HNO_3 from individual commercial stock solutions of Al, As, Cd, Co, Cr, Cu, Fe, Mg, Mn, Ni, Pb, Sn, V, and Zn (1000 $mg \cdot L^{-1}$ in 5% HNO_3, Specpure from Alfa Aesar, War Hill, MA). Be^{9}, Sc^{45}, In^{115}, and Bi^{209} from stock standard solutions (1000 $mg \cdot L^{-1}$ in 5% HNO_3, Specpure), were added to blank, standard and sample solutions as internal standard to yield 10 $\mu g \cdot L^{-1}$. Argon (99.999%, Praxair, Spain) was used as plasmogen and carrier gas. A certified reference material NIST-SRM1646a (estuarine sediment from the National Institute of Standards and Technology, Gaithersburg, MD) was used to validate the accuracy of the whole procedure.

2.2. Samples and Measurements

117 sediment samples were collected from the estuary of the Nerbioi-Ibaizabal River, located on the continental shelf of the Cantabrian coastline in the south-eastern part of the Bay of Biscay. Approximately 500 g of surface sediment samples (~2 cm depth) were collected at low tide, from eight stations distributed with an average distance of 11km (see Figure 1).

Two sampling points (Nos. 1, 2) are located in the main channel, four (Nos. 3, 4, 5, 7) in the main tributary rivers, another one (No. 8) in the mouth of the estuary, and one (No. 6) in a semiclosed dock. Sediments were sampled every three months (15 sampling campaigns from January 2005 to October 2008), using plastic sampling utensils and latex gloves to avoid sample contamination. Samples were set inside cleaned plastic bags and transported to the laboratory at 4 °C to reduce the microbiological activity (*17*). Sediment samples were frozen at -20 °C, lyophilized at 150 mTorr and -52 °C in a Cryodos apparatus (Telstar, Spain) for 48 h and then sieved until a maximum particle size of 63 μm and kept in the refrigerator at 4 °C. Trace elements were determined in the fine fraction (<63 μm) of the sediments by ICP/MS after acid extraction with HNO_3 and HCl accelerated with focused ultrasound energy (*18*).

Two mL standard glass chromatographic vials (12 × 32 mm) of 9.5 mm internal diameter were used as measurement cells to collect diffuse reflectance spectra in the NIR from 2500 to 800 nm (4000 to 12500 cm^{-1})

using a resolution of 4 cm^{-1}, 36 cumulated scans per spectrum and a background of the closed integrating sphere were employed.

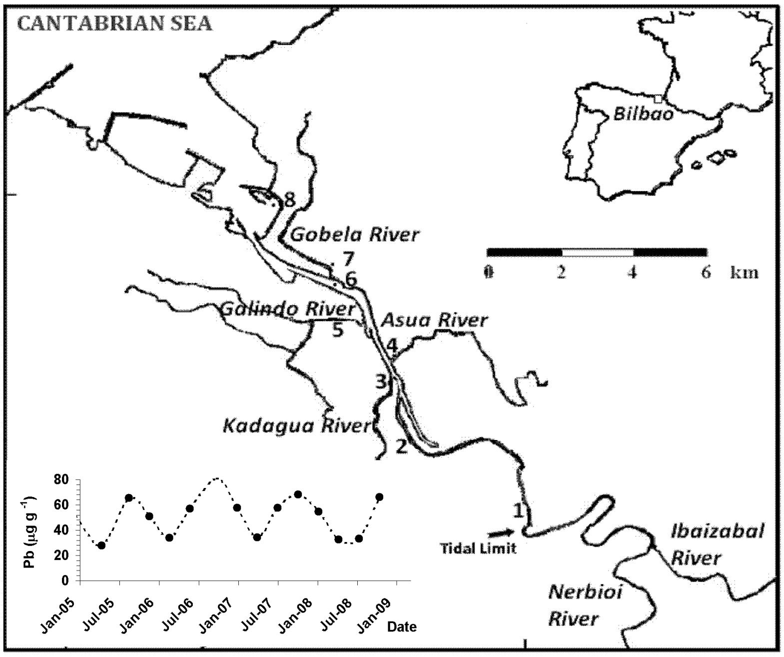

Figure 1. Geographical location of the Nerbioi-Ibaizabal estuary (Metropolitan Bilbao) and spatial distribution of the different sampling stations: 1. Alde Zaharra (*AZ*), 2. Zorroza (*ZO*), 3. Kadagua (*KA*), 4. Asua (*AS*), 5. Galindo (*GA*), 6. Udondo (*UD*), 7. Gobela (*GO*), and 8. Arriluze (*AR*). Lead concentration profile as the average total concentration of *AZ*, *ZO*, and *KA* sampling stations among the 15 sampling campaigns.

The spectra in the MIR, between 4000 and 600 cm^{-1}, were obtained placing few mg of sediment onto the attenuated total reflectance (ATR) accessory, using 4 cm^{-1} resolution, 36 scans and a background for the empty ATR plate cell. Blank spectra were scanned after the triplicate measurement of each sample, for assessing the absence of cross-contamination after cleaning the ATR crystal and a new background was collected again. It must be noticed that on considering the variability of ATR spectra, average spectra were used for further calculations, instead of using each replicate in a separate way. The aforementioned spectra were treated by chemometrics.

Clustering analysis of the sediments spectra was used as in previous works (*15, 19*) to evaluate the variability of samples and the selection of calibration and validation data sets. Appropriate partial least-squares (PLS) multivariate models were built from the calibration set, after testing several spectral windows combined with different preprocessing algorithms, and selecting the optimum number of PLS factors from the minimum of the predicted residual error sum of squares (PRESS). Concerning the spectral window selection, two optimization approaches, the synergy interval PLS (siPLS) (*20*) and backward interval PLS (biPLS) (*21*) methods, were used for both, NIR and MIR, data.

In the present work, five preprocessing methods were employed: (i) the constant offset elimination (COE) which shifts the spectra in order to set the *y*-minimum to zero; (ii) the straight line subtraction (SLS) which fits a straight line to the spectrum and subtracts it; (iii) the vector normalization (VN) which carries out a mean centering followed by a variance scaling; (iv) the multiplicative scatter correction (MSC) which performs a linear transformation of each spectrum to match the average spectrum of the whole set; and (v) the differentiation by calculating the first derivative (FD) (*22*).

Prediction accuracy and predictive ability of the PLS models were evaluated in samples not used for calibration by employing the root-mean-square error of prediction (RMSEP) and the residual predictive deviation (RPD), defined as the ratio between the standard deviation of the population (SD) in the validation set and the RMSEP (*23, 24*). A relatively small RPD indicates a low robust PLS calibration model while the increase of the value of RPD means an improvement of the prediction power. Various quality indicators, as the mean difference (*dx*-*y*) between the predicted values and the reference data (*Ci*), and the standard deviation of the mean difference (*sx*-*y*), as well as *s*trip, the deviation between triplicate determinations, were also used (*24*). In spite that several spectral windows as well as spectral preprocessing algorithms were tested for evaluating the prediction capabilities of the corresponding models, only the most significant results are presented through this paper.

3. Results and Discussion

Metal concentrations obtained by ICP-MS for samples considered in this study ranged between 900 and 15500 mg·kg^{-1} for Al, from 0.6 to 220 mg·kg^{-1} for As, from non detected to 18 mg·kg^{-1} for Cd, between 0.3 and 32 mg·kg^{-1} for Co, from 5 to 175 mg·kg^{-1} for Cr, between 15 and 575 mg·kg^{-1} for Cu, from 5200 to 36720 mg·kg^{-1} for Fe, between 330 and 8340 mg·kg^{-1} for Mg, from 65 to 935 mg·kg^{-1} for Mn, between 3.3 and 320 mg·kg^{-1} for Ni, from

21 to 445 mg·kg^{-1} for Pb, from non detected to 68 mg·kg^{-1} and 37 mg·kg^{-1} for Sn and V, respectively, and between 40 and 2060 mg·kg^{-1} for Zn. So, once again it is clear that screening of the aforementioned elements by IR spectroscopy must be based on the modelization of their interaction with the organic matter and not at all on their specific bands.

As an example of the evolution of trace element content, Figure 1 includes the average total concentration of Pb found during the 15 sampling campaigns in the three sampling stations closest to Bilbao city (*AZ*, *ZO*, and *KA*) where the impacts from the industrial activity have decreased to the minimum (*17*). A seasonal trend has been observed during these four years of continuous monitoring, showing a decreasing of lead content in the sediment in autumn-winter and an increasing in spring-summer. The same like a wave trend was observed for As, Cu, Zn, and Sn, with maximum and minimum concentrations in the same period of the year. Maybe this trend reflects the impact of a continuous low level pollution in this part of the estuary, being the leaching from the city (soils, buildings, urban ware, etc.) and the mineral weathering within the basin the main sources for the input of such elements. The aforementioned considerations will be of a great importance to explain that IR models were based on the trace element-organic matter interaction and not on the different organic composition of the sampling sites.

3.1. Estuarine Sediment IR Spectra

All sediments tested in this study provided similar vibrational reflectance spectra. Figure 2A shows the assembled spectra of samples in the NIR from 2500 to 800 nm (4000 to 12 500 cm^{-1}). All spectra have three major absorption peaks around 2209, 1925, and 1414 nm. Reflectance characteristics of soils are related to chemical groups present in the organic matter, being chlorophyll, oil, cellulose, pectin, starch, lignin, and humic acids the spectrally active groups in this region (*25*). In general, soil reflectance decreases on decreasing the organic matter content (*26*). Band placed at 2209 nm could be related to C—H absorption and combination bands while those located at 1925 and 1414 nm could be related with O—H bands. Additionally few small bands located around 2250 and 2450 nm, can be also identified in all the samples considered. Among them, that present at 2352 nm could be associated to the absorption of C—H and CH_2 (from cellulose). On regarding the MIR from 4000 to 600 cm^{-1} all FTIR-ATR collected spectra for sediment samples showed similar features (Figure 2B).

As it can be seen, sediments exhibit their main bands in the region below 1700 cm^{-1}. The band at 1645 cm^{-1} is mainly attributed to free water. However in our case sediment samples were frozen and lyophilized and thus not contain water as it can be confirmed by the absence of the band at 3500 cm^{-1}. So, the aforementioned band can be due to the C=C stretching of aromatics, alkene double bonds (*27*), amide C=O group or COO asymmetric stretching of metal carboxylates (*28*), as indicated in previous works.

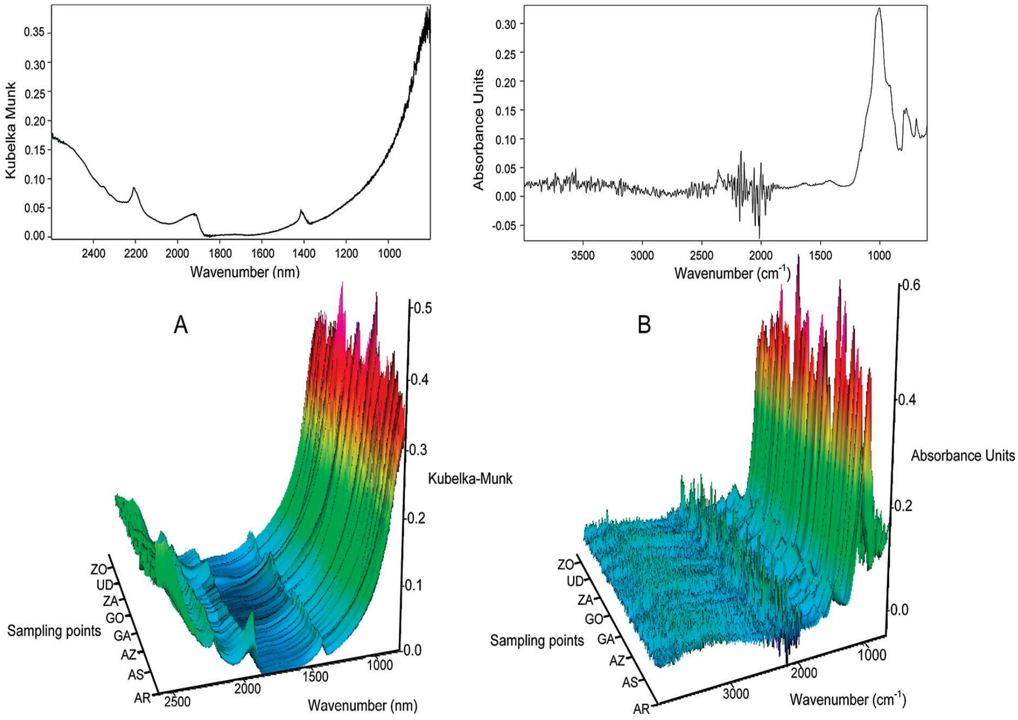

Figure 2. Assembled reflectance spectra of all estuarine sediment samples from the eight sampling points (ordered according to the seasonal period inside of each sampling point) in the NIR (A) and MIR (B) domains, respectively. Instrumental conditions: 4 cm^{-1} nominal resolution and 36 cumulated scans per spectrum. Spectra corresponding to sample AS_0603 have been presented alone for a clear identification of vibrational bands in both domains.

Bands located at 1432, 876, and 712 cm^{-1} are associated to the presence of calcite which is coherent with Raman spectroscopy studies of river sediments in the Basque Country (*29*). Additional bands placed at 797, 778, and 693cm^{-1} could be caused by other carbonates (Li+, K+, Na+, …) (*30*) which could overlap some bands corresponding to silicates. Additionally, the band at 778 cm^{-1} can be also related to the Fe(II)—OH group and that located at 693 cm^{-1} to quartz (*28*). These compounds can also show absorption bands at 1165, 1083, and 1030 cm^{-1} (*31*). The most intense band around 1011 cm^{-1} could be considered as a Si—O stretching band (*28*), and the shoulder exhibited at 914 cm^{-1} can be related to the presence of $Al(OH)_3$.

In spite of the fact that intense bands in the NIR or MIR spectra are not directly associated to the presence of trace elements per se, it is clear that metals can interact with the main sediment components, specially the organic ones acting as complexing ligands. Based on this phenomenon it can be developed calibration models from previously analyzed samples for screening purposes (*27, 28*).

3.2. Cluster Classification of Sediment Samples

A hierarchical clustering method was applied before multivariate data treatment to afford the proper selection of a representative calibration set containing all the variability of samples under study and thus improving the prediction capabilities. From previous experiences (*15, 16, 24*) we selected dendrogram classification using Euclidean distance with Ward linkage upon considering the frequency range between 2500 and 1000 nm for NIR and two spectral ranges compressed from 4000 to 2384 and from 1937 to 600cm^{-1} for MIR, both applied on the vector normalized spectral data. SI Figure S1 (see Supporting Information) shows the dendrographic classification of samples in NIR (S1A) and MIR (S1B) domains and provides 6 and 12 different types of samples for cutoff values of 0.75 and 0.35, respectively. The main groups of clusters formed are directly correlated with the intensity of the vibrational bands of sediments, being samples with high reflectance level grouped together (for details about mean and the standard deviation values of sediment metal contents for the clusters see SI Table S2). In spite that trace elements do not show absorption features in the infrared region, it is possible to detect from data reported (see SI Table S2, in which ± s refers to the standard deviation of the mean and all metal concentration values are expressed in $\mu g \cdot g^{-1}$ units) some correlations between clusters structure and trace metal content of sediments as they may be detectable because of their possible adsorption to clays that absorb light in this wavelength range (*32*) or their complexation with organic matter or associated with carbonates, silicates, hydroxides, sulphides, or oxides (*33*). These correlations are better defined in the NIR domain.

The six groups of samples in the NIR dendrographic classification have the same elements (Fe, As, Zn, V, Sn, Pb, Cu, and Cr) ordered in a systematic way (see SI Table S2). Cluster index 2 and 1 are formed by group of samples with the highest concentration (1st and third ranking respectively) in those elements but covering only spring-summer-autumn campaigns in which the maximum seasonal concentration is observed (see the seasonal trend for Pb in Figure 1).

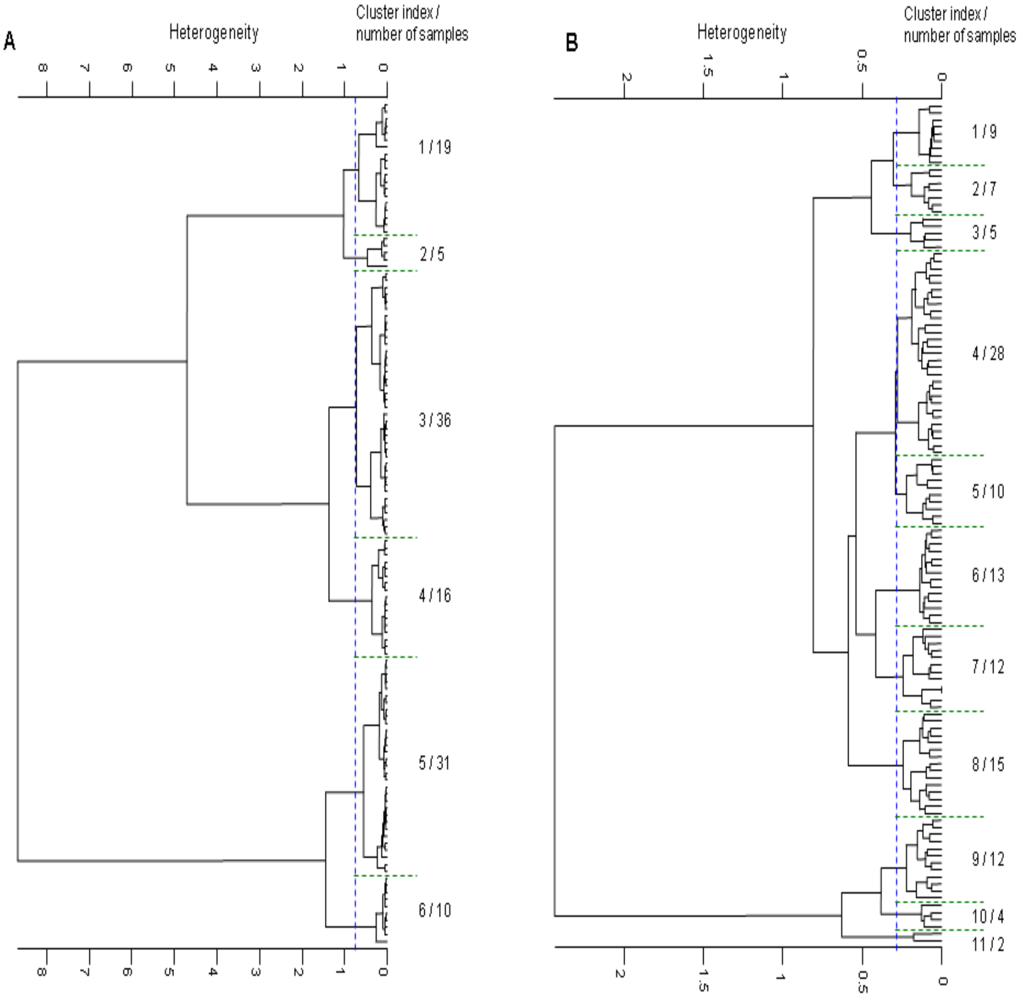

Figure S1. Dendrographic classification of sediment samples from their NIR (S1A) and MIR (S1B) reflectance spectra. The Euclidean distance on the vector normalized spectra and the Ward linkage methods were used. For details about cluster group composition see data in Table S2. Samples are identified with two letters (corresponding to the sampling point) and 4 numbers (the first two numbers for the year and the last ones corresponding to the month).

Table S2. Characteristics of estuarine sediment samples classified into clusters after dendrographic treatment of reflectance data.

Sampling technique			**NIR**					
Cluster index			**1**	**2**	**3**	**4**	**5**	**6**
Number of samples			**19**	**5**	**36**	**16**	**31**	**10**
Metals	**[Al]**	**mean ± s**	6537 ± 3311	4189 ± 1418	4354 ± 1780	4610 ± 1581	3566 ± 1936	2350 ± 1158
	[As]	**mean ± s**	64 ± 59	144 ± 85	31 ± 31	79 ± 47	17 ± 20	13 ± 27
	[Cd]	**mean ± s**	5 ± 4	6 ± 5	3 ± 3	4 ± 3	1.6 ± 2.5	0.4 ± 1.3
	[Co]	**mean ± s**	8 ± 3	9 ± 2	7 ± 2	9 ± 6	6 ± 3	5 ± 2
	[Cr]	**mean ± s**	54 ± 19	65 ± 17	42 ± 23	56 ± 33	30 ± 13	18 ± 9
	[Cu]	**mean ± s**	184 ± 112	297 ± 59	113 ± 98	259 ± 167	84 ± 100	77 ± 92
	[Fe]	**mean ± s**	18886 ± 7844	26194 ± 7837	12298 ± 3288	19985 ± 6877	11157 ± 6119	8658 ± 3207
	[Mg]	**mean ± s**	3633 ± 2140	2247 ± 1382	3065 ± 1693	3559 ± 1799	2471 ± 1379	2305 ± 2759
	[Mn]	**mean ± s**	488 ± 262	302 ± 200	299 ± 59	403 ± 215	256 ± 149	21 7 ± 51
	[Ni]	**mean ± s**	31 ±24	25 ± 7	27 ± 17	50 ± 77	25 ± 21	14 ± 7
	[Pb]	**mean ± s**	206 ± 95	338 ± 75	143 ± 87	251 ± 77	107 ± 93	72 ± 8 6
	[Sn]	**mean ± s**	24 ± 11	42 ± 13	15 ± 8	31 ± 13	10 ± 8	6 ± 6
	[V]	**mean ± s**	16 ± 11	27 ± 10	9 ± 7	18 ± 7	6 ± 7	4 ± 8
	[Zn]	**mean ± s**	890 ± 438	1087 ± 360	523 ± 270	858 ± 336	397 ± 355	358 ± 390
Samples			AR_0505, AR_0509, AR_0603, AR_0607, AR_0707, AR_0807, AS_0505, GA_0501, GA_0512, GA_0603, GA_0610, GA_0707, GO_0512, GO_0603, KA_0707, UD_0512, UD_0607, UD_0610, UD_0707	GA_0505, GO_0801, UD_0501, UD_0505, UD_0509	AR_0512, AR_0610, AR_0701, AR_0704, AR_0710, AR_0801, AR_0810, AS_0501, AS_0509, AS_0512, AS_0603, AS_0607, AS_0701, AS_0707, AS_0810, AZ_0501, AZ_0610, AZ_0701, AZ_0710, AZ_0801, GA_0701, GA_0807, GO_0607, GO_0804, GO_0807, KA_0701, KA_0710, KA_0801, KA_0804, UD_0704, UD_0804, UD_0807, ZO_0501, ZO_0512, ZO_0610, ZO_0701	AR_0804, GA_0607, GA_0704, GA_0804, GA_0810, GO_0501, GO_0505, GO_0509, GO_0701, GO_0707, UD_0603, UD_0701, UD_0710, UD_0801, UD_0810, ZO_0801	AS_0610, AS_0704, AS_0710, AS_0801, AS_0804, AS_0807, AZ_0509, AZ_0512, AZ_0603, AZ_0707, AZ_0807, AZ_0810, GA_0509, GA_0710, GA_0801, GO_0610, GO_0704, GO_0710, KA_0501, KA_0509, KA_0512, KA_0610, KA_0704, KA_0807, ZO_0505, ZO_0509, ZO_0603, ZO_0607, ZO_0707, ZO_0710, ZO_0804, ZO_0807, ZO_0810	AZ_0505, AZ_0607, AZ_0704, AZ_0804, GO_0810, KA_0505, KA_0603, KA_0607, KA_0810, ZO_0704

Table S2 (continued). Characteristics of estuarine sediment samples classified into clusters after dendrographic treatment of reflectance data.

Sampling technique			**MIR**					
Cluster index			**1**	**2**	**3**	**4**	**5**	**6**
Number of samples			**9**	**7**	**5**	**28**	**10**	**13**
Metals	**[Al]**	**mean ± s**	4642 ± 4216	2003 ± 667	8814 ± 4691	4336 ± 1615	4488 ± 1998	6401 ± 2600
	[As]	**mean ± s**	15 ± 16	4 ± 3	32 ± 16	59 ± 68	25 ± 51	46 ± 39
	[Cd]	**mean ± s**	0,7 ± 1,1	0,1 ± 0,2	1.5 ± 1.5	4 ± 4	0.9 ± 1.5	3 ± 4
	[Co]	**mean ± s**	5 ± 3	4,1 ± 1,4	7 ± 3	8 ± 3	4.5 ± 1.3	6 ± 3
	[Cr]	**mean ± s**	22 ± 10	20 ± 10	28 ± 11	52 ± 19	32 ± 12	34 ± 19
	[Cu]	**mean ± s**	76 ± 53	86 ± 77	71 ± 28	148 ± 93	64 ± 54	214 ± 193
	[Fe]	**mean ± s**	9125 ± 3652	8624 ± 2232	12005 ± 2751	16388 ± 7895	10146 ± 3471	13691 ± 4909
	[Mg]	**mean ± s**	2811 ± 2691	1002 ± 814	5577 ± 2673	2429 ± 1270	1579 ± 477	3114 ± 1014
	[Mn]	**mean ± s**	326 ± 213	218 ± 64	527 ± 249	290 ± 170	159 ± 55	310 ± 165
	[Ni]	**mean ± s**	12 ± 6	12 ± 7	13 ± 3	34 ± 28	20 ± 4	20 ± 9
	[Pb]	**mean ± s**	68 ± 49	37 ± 17	125 ± 28	208 ± 117	82 ± 77	164 ± 91
	[Sn]	**mean ± s**	8 ± 6	4 ± 3	15 ± 4	23 ± 14	11 ± 9	17 ± 11
	[V]	**mean ± s**	6 ± 6	2 ± 3	11 ± 6	13 ± 12	5 ± 10	15 ± 10
	[Zn]	**mean ± s**	388 ± 394	140 ± 67	760 ± 467	707 ± 441	524 ± 455	59 7 ± 453
Samples			AR_0505, AR_0509, AR_0512, AS_0501, GA_0512, GO_0505, GO_0509, GO_0512, KA_0501	AZ_0505, AZ_0509, AZ_0512, KA_0505, KA_0512, ZO_0505, ZO_0509	AR_0603, AR_0607, AR_0610, AR_0701, KA_0607	AR_0704, AS_0505, AS_0509, AS_0512, AS_0704, AS_0710, AS_0810, AZ_0501, AZ_0701, AZ_0710, GA_0501, GA_0505, GA_0701, GA_0704, GA_0707, GA_0710, KA_0701, UD_0501, UD_0505, UD_0509, UD_0512, UD_0701, UD_0707, UD_0710, ZO_0501, ZO_0512, ZO_0701, ZO_0710	AS_0707, AZ_0607, AZ_0610, AZ_0704, AZ_0707, UD_0610, ZO_0607, ZO_0610, ZO_0704, ZO_0707	AR_0707, AR_0710, GO_0607, GO_0610, GO_0701, GO_0704, GO_0707, GO_0710, KA_0610, KA_0704, KA_0707, KA_0710, UD_0704

Table S2 (continued). Characteristics of estuarine sediment samples classified into clusters after dendrographic treatment of reflectance data.

Sampling technique			MIR				
Cluster index			**7**	**8**	**9**	**10**	**11**
Number of samples			12	15	12	4	2
Metals	**[Al]**	**mean ± s**	5075 ± 1120	2403 ± 1535	3833 ± 1297	3863 ± 1082	1106 ± 274
	[As]	**mean ± s**	74 ± 60	30 ± 38	27 ± 32	9 ± 10	40 ± 54
	[Cd]	**mean ± s**	5 ± 3	2.2 ± 1.6	2 ± 3	0.6 ± 1.1	3 ± 4
	[Co]	**mean ± s**	7 ± 3	11 ± 6	8 ± 3	4.5 ± 1.8	7 ± 4
	[Cr]	**mean ± s**	52 ± 12	43 ± 38	43 ± 31	20 ± 11	34 ± 10
	[Cu]	**mean ± s**	224 ± 131	104 ± 89	107 ± 102	34 ± 19	94 ± 103
	[Fe]	**mean ± s**	18418 ± 6020	12424 ± 6490	12474 ± 3916	7811 ± 729	11021 ± 3491
	[Mg]	**mean ± s**	2962 ± 690	5068 ± 2135	3131 ± 2049	960 ± 230	2493 ± 2182
	[Mn]	**mean ± s**	366 ± 253	315 ± 172	329 ± 163	282 ± 131	194 ± 81
	[Ni]	**mean ± s**	29 ± 16	56 ± 77	29 ± 17	13 ± 6	20 ± 3
	[Pb]	**mean ± s**	250 ± 79	153 ± 108	109 ± 68	67 ± 67	97 ± 107
	[Sn]	**mean ± s**	28 ± 10	16 ± 16	13 ± 7	3.0 ± 0.5	14 ± 13
	[V]	**mean ± s**	17 ± 10	10 ± 7	10 ± 8	1 ± 2	9 ± 13
	[Zn]	**mean ± s**	927 ± 362	479 ± 295	458 ± 257	341 ± 300	430 ± 448
Samples			AR_0801, AS_0607, AS_0610, GA_0603, GA_0607, GA_0801, GO_0501, GO_0603, GO_0801, UD_0603, UD_0607, UD_0801	AR_0810, AS_0701, AS_0807, AZ_0807, AZ_0810, GA_0610, GA_0804, GA_0807, GA_0810, GO_0810, KA_0807, KA_0810, UD_0810, ZO_0807, ZO_0810	AR_0804, AR_0807, AS_0801, AS_0804, AZ_0801, GO_0804, GO_0807, KA_0801, KA_0804, UD_0804, ZO_0801, ZO_0804	AS_0603, AZ_0603, KA_0603, ZO_0603	AZ_0804, UD_0807

Cluster index 4 and 3 are formed by samples with intermediate concentration (2nd and fourth ranking respectively) in those elements including only the autumn-winter-spring campaigns where the minimum seasonal concentrations are measured in the sediments. Cluster index 5 and 6 are not related to seasonality and correlate only with the lowest (5th and 6th respectively) rank of concentrations for those elements. So, for the most important elements, the NIR clustering is related to seasonal trends in the concentration profiles

3.3. Selection of the Calibration Set

To built calibration models 25 and 33 samples were selected for NIR and MIR, respectively, from the information provided by SI Figure S1 and predictive capabilities and analytical features of models were established then using the rest, 92 and 84 samples, respectively. An additional criterion, based on an extended number of samples for calibration obtained from the rounded value of a half sample number from each cluster, was used for comparative purposes. With this new criterion, PLS models were built using 58 and 62 samples for NIR and MIR and evaluated through the predictive capabilities obtained in the rest 59 and 55 samples, for NIR and MIR domains respectively. Samples were always randomly selected from each cluster, but trying to avoid the selection of samples from the same location and seasonal sampling campaign.

3.4. Estimation of Metal Content from NIR Spectra

Total contents of Al, As, Cd, Co, Cr, Cu, Fe, Mg, Mn, Ni, Pb, Sn, V, and Zn, were predicted using PLS-NIR models previously described. As an example, Figure 3 shows the comparison between the calibration and validation sets for Arsenic, as an element with low concentration, and Iron, as an element with high concentration, in all the samples. As can be seen, the calibration and validation correlation lines are coincident.

Table 1 reports the optimized conditions as well as prediction capabilities obtained for elements considered using the aforementioned sets. As it can be seen, significant differences in the optimum spectral range selected for each element (from single to four regions combination) and the number of extracted factors (between 1 and 9) were found for the different PLS models built. For a critical comparison between models, it must be noticed that when the extended criterion was used, better predictive capabilities (RPD values ranging from 1.3 to 2.6) than those achieved using the normal ones (RPD values between 1.3

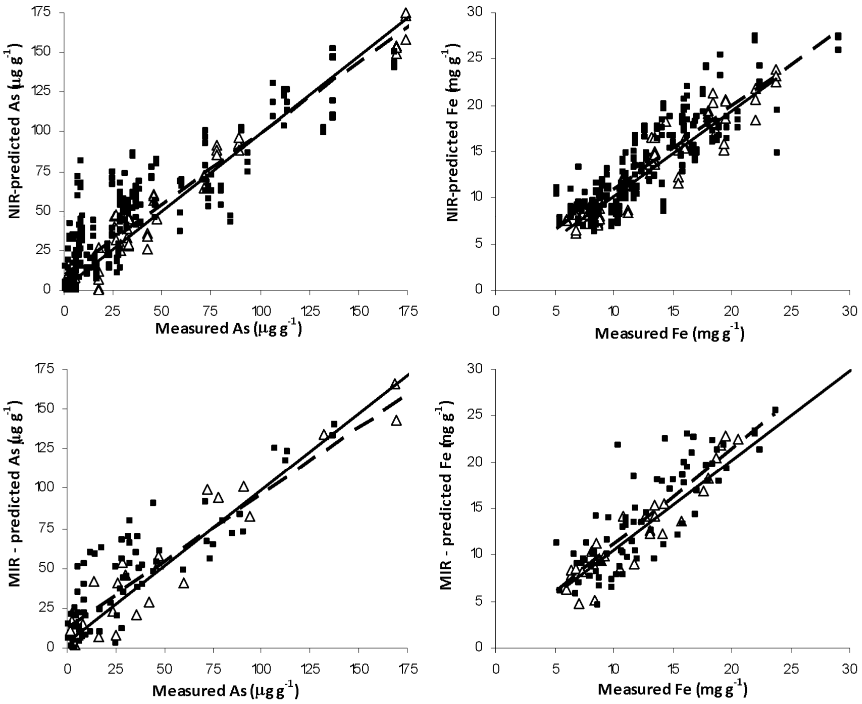

Figure 3. Comparison the results of calibration (continued line) and validation (dashed line) like predicted versus observed of As and Fe concentrations in sediment from Nerbioi-Ibaizabal estuary for the normal models in NIR and MIR, respectively. Concentration values are expressed in μg·g^{-1} for As and mg·g^{-1} for Fe. ▶: calibration samples, ■:: validation samples.

and 2.2), were obtained with the exception of Mg and Mn. Additionally, for Co and Ni, low predictive ability was obtained using the extended calibration set. The bad prediction results obtained for the two aforementioned metals are probably link to the presence of high proportions of both metals as inorganic forms without a remarkable influence in the NIR spectra and on the basis that many of the elements predicted are indirectly modelized and not based on their NIR behaviour. In fact, the different behaviour of Ni and Co against Al was the basis to develop a separation process in the presence of sulphate (*34*). The same statements can be extracted for the comparison of regression values (*r*), thus indicating, in general terms, an appropriate predictive capability for screening purposes. In the case of As, Pb, and Sn, the use of NIR measurements provides good prediction capability to quantify these elements in sediments.

Table 1. Prediction capabilities of PLS-NIR for the determination of metals in estuarine sediment samples from Nerbioi-Ibaizabal River.

Criterion	**Metal**	**Wave number range (nm)**	**Spectral pre-processing**	**PLS factors**	**r**	**RMSEC (µg·g^{-1})**	**RMSEP ((µg·g^{-1})**	$d_{(x-y)}$ **((µg·g^{-1})**	s_{trip} **((µg·g^{-1})**	***RPD***
Normal	Al	2241-1970 / 1758-1002	None	5	0.79	910	1515	227	261	1.6
	As	2598-1067	MSC	7	0.86	10	25	3	5	1.9
	Cd	2598-2241 / 1587-1328	MSC	6	0.68	1.0	2.5	0	0.5	1.4
	Co	2598-2241 / 1142-1067	MSC	3	0.47	1.4	3.4	1	0.5	1.1
	Cr	2598-2241 / 1970-1758 / 1587-1446 / 1228-1142	MSC	1	0.63	11	19	-3	1.3	1.3
	Cu	2241-1758 / 1587-1446 / 1328-1142	None	5	0.81	39	76	7	12	1.7
	Fe	1836-1333	VN	4	0.86	1951	3602	418	776	1.9
	Mg	2354-2173 / 1836-1731	VN	3	0.70	1306	1381	-307	164	1.4
	Mn	2598-2241 / 1970-1587 / 1446-1328	None	9	0.75	43	133	8	25	1.5
	Ni	2598-2241 / 1446-1328	None	5	0.50	14	30	0	3	1.1
	Pb	2260-2173 / 1732-1639	COE	4	0.81	42	68	-20	12	1.6
	Sn	2354-2073 / 1854-1675 / 1406-1063	VN	4	0.90	4	6	-1	1.1	2.2
	V	2354-2073 / 1676-1002	MSC	2	0.80	4	6	1	0.9	1.6
	Zn	1676-1405	None	4	0.66	125	318	-2	52	1.3

Note: r means the regression coefficient; RMSEC is root mean square error of calibration; RMSEP is root mean square error of prediction; $d_{(x-y)}$ is the mean difference between predicted versus actual metal content value, respectively; s_{trip} is the standard deviation of three replicates. RPD is the residual predictive deviation. Spectral pre-processing significance is detailed on the body of the text.

Table 1 (continued). The same explanation as indicated in Table 1.

Criterion	**Metal**	**Wave number range (nm)**	**Spectral pre-processing**	**PLS factors**	**r**	**RMSEC (μg·g^{-1})**	**RMSEP (μg·g^{-1})**	$d_{(x-y)}$ **(μg·g^{-1})**	s_{trip} **(μg·g^{-1})**	***RPD***
Extended	Al	2241-1970 / 1758-1002	None	6	0.76	1217	1313	-90	461	1.5
	As	2598-1067	MSC	6	0.92	20	19	4	6	2.6
	Cd	2598-2241 / 1587-1328	MSC	4	0.76	2.7	2.1	0	0.4	1.5
	Co	2598-2241 / 1142-1067	MSC	1	0.48	3.9	2.3	1	0.3	0.9
	Cr	2598-2241 / 1970-1758 / 1587-1446 / 1228-1142	MSC	1	0.81	22	11	2	1.9	1.7
	Cu	2241-1758 / 1587-1446 / 1328-1142	None	5	0.85	74	66	10	16	1.9
	Fe	1836-1333	VN	3	0.88	3042	3837	-1184	949	1.9
	Mg	2354-2173 / 1836-1731	VN	6	0.64	899	1335	-43	496	1.3
	Mn	2598-2241 / 1970-1587 / 1446-1328	None	9	0.72	98	133	-24	28	1.4
	Ni	2598-2241 / 1446-1328	None	1	0.06	43	19	7	4	0.8
	Pb	2260-2173 / 1732-1639	COE	5	0.88	60	50	13	13	2.0
	Sn	2354-2073 / 1854-1675 / 1406-1063	VN	5	0.92	5	5	-1	1.4	2.5
	V	2354-2073 / 1676-1002	MSC	4	0.81	4	6	1	1.2	1.7
	Zn	1676-1405	None	3	0.77	351	239	53	37	1.5

Table 2. Prediction capabilities of PLS-MIR for the determination of metals in estuarine sediment samples from Nerbioi-Ibaizabal River.

Criterion	**Metal**	**Wave number range (cm^{-1})**	**Spectral pre-processing**	**PLS factors**	**r**	**RMSEC (μg·g^{-1})**	**RMSEP (μg·g^{-1})**	**$d_{(x-y)}$ (μg·g^{-1})**	***RPD***
Normal	Al	1663-1395 / 1263-997	FD + MSC	4	0.78	1270	1385	79	1.6
	As	1796-1395 / 1132-600	None	7	0.85	17	26	8	1.8
	Cd	1927-1661 / 1132-997 / 866-600	COE	4	0.71	2.1	2.5	0	1.4
	Co	1263-997	FD + SLS	4	0.48	1.8	3.4	0	1.1
	Cr	1397-1263 / 999-864 / 733-600	None	3	0.61	19	19	-1	1.3
	Cu	1663-864	MSC	7	0.79	77	77	5	1.6
	Fe	1132-997 / 866-731	None	7	0.83	3164	3946	400	1.8
	Mg	1796-1661 / 1397-1130 / 999-731	FD + VN	5	0.78	834	1112	24	1.6
	Mn	1796-1528 / 1263-1130 / 866-600	None	5	0.74	140	138	51	1.4
	Ni	1796-1661 / 1132-997 / 866-600	COE	5	0.56	9	33	-5	1.2
	Pb	1927-1661 / 1132-997 / 866-600	COE	4	0.82	55	63	4	1.7
	Sn	1663-1528 / 1397-1263 / 1132-864 / 733-600	COE	3	0.83	4	7	0	1.8
	V	1796-1528 / 1263-600	COE	7	0.86	3	5	1	2.0
	Zn	1927-1263 / 1132-997 / 866-600	COE	5	0.69	172	312	-30	1.4

Note: The meaning of evaluated parameters as indicated in the footnote of Table 1.

Table 2 (continued). The same explanation as indicated in Table 2.

Criterion	**Metal**	**Wave number range (cm^{-1})**	**Spectral pre-processing**	**PLS factors**	**r**	**RMSEC (μg·g^{-1})**	**RMSEP (μg·g^{-1})**	**$d_{(x-y)}$ (μg·g^{-1})**	***RPD***
Extended	Al	1663-1395 / 1263-997	FD + MSC	2	0.76	1677	1408	365	1.5
	As	1796-1395 / 1132-600	None	7	0.88	20	26	8	2.0
	Cd	1927-1661 / 1132-997 / 866-600	COE	2	0.72	2.7	2.5	0	1.4
	Co	1263-997	FD + SLS	2	0.45	2.8	3.9	0	1.1
	Cr	1397-1263 / 999-864 / 733-600	None	2	0.54	19	21	1	1.2
	Cu	1663-864	MSC	7	0.82	64	88	-21	1.6
	Fe	1132-997 / 866-731	None	7	0.82	3911	4048	1001	1.7
	Mg	1796-1661 / 1397-1130 / 999-731	FD + VN	4	0.75	978	1190	54	1.5
	Mn	1796-1528 / 1263-1130 / 866-600	None	5	0.79	125	125	14	1.6
	Ni	1796-1661 / 1132-997 / 866-600	COE	4	0.50	15	40	-5	1.1
	Pb	1927-1661 / 1132-997 / 866-600	COE	3	0.83	68	62	11	1.8
	Sn	1663-1528 / 1397-1263 / 1132-864 / 733-600	COE	2	0.83	6	8	0	1.8
	V	1796-1528 / 1263-600	COE	5	0.90	4	5	1	2.3
	Zn	1927-1263 / 1132-997 / 866-600	COE	3	0.76	317	266	77	1.5

As compared with previously reported procedures (see SI Table S1) it can be observed that RPD values found through this study (in the NIR domain) are of a similar order than those previously found for these elements.

3.5. Estimation of Metal Content from MIR Spectra

The studied elements were modelized using MIR spectra. Figure 3 shows for comparison the plots of calibration and validation sets obtained with MIR data for As and Fe. Optimized spectral conditions and analytical features achieved for each one of the elements under study are reported in Table 2. From single to four combined spectral regions, extracting between 2 and 7 PLS factors, were needed to build appropriate PLS models in the MIR range. Constant offset elimination algorithm seems to be the best spectral preprocessing algorithm when working with the ATR spectra. From results in Table 2 it can be appreciated that differences in predictive ability between the different assayed criteria are fairly small. Using MIR data, bad predictive results were obtained for Co and Ni, thus enforcing the statement previously indicated for NIR data. From the comparison of results achieved using NIR and MIR data it can be concluded that NIR based PLS models seem to be more consistent and robust and well related to the seasonality found for the total element concentrations among campaigns. However both kind of signals are useful to develop fast green alternatives for screening trace elements in sediments.

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Partial least squares X-ray fluorescence determination of trace elements in sediments from the estuary of Nerbioi-Ibaizabal River

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Partial least squares X-ray fluorescence determination of trace elements in sediments from the estuary of Nerbioi-Ibaizabal River

Abstract

The feasibility of partial least-squares (PLS) regression modelling of X-ray fluorescence (XRF) spectra of estuarine sediments has been evaluated as a tool for rapid trace element content monitoring. Multivariate PLS calibration models were developed to predict the concentration of Al, As, Cd, Co, Cr, Cu, Fe, Mg, Mn, Ni, Pb, Sn, V and Zn in sediments collected from different locations across the estuary of the Nerbioi-Ibaizabal River (Metropolitan Bilbao, Bay of Biscay, Basque Country). The study was carried out on a set of 116 sediment samples, previously lyophilized and sieved with a particle size lower than 63 µm. Sample reference data were obtained by inductively coupled plasma mass spectrometry. 34 samples were selected for building PLS models through a hierarchical cluster analysis. The remaining 82 samples were used as a test set to validate the models. Results obtained in the present study involved relative root mean square errors of prediction varying from 21%, for the determination of Pb at hundreds $\mu g \cdot g^{-1}$ level, up to 87%, for Ni determination at little tens $\mu g \cdot g^{-1}$ level. An average prediction error of ± 37% for the 14 elements under study was obtained, being in all cases mean differences between predicted and reference results of the same order than the standard deviation of three replicates from a same sample.

Residual predictive deviation values obtained ranged from 1.1 up to 3.9.

Keywords: Partial-least-squares; X-ray fluorescence, estuarine sediments, metals, trace elements.

1. Introduction

Estuaries suffers from a series of contaminant inputs of a variety of industrial and domestic sources including sewage out falls, agricultural and road run-off, atmospheric deposition and pollution from shipping [1]. Their sediments show a great capacity to integrate and accumulate trace elements from low concentrations in the overlying water column [2]. Trace elements are serious pollutants due to their toxicity, persistence, and non-degradability in the environment [3] and, because of that, assessment of sediment contamination from metals must be carefully controlled to prevent their adverse effects on aquatic organisms, including those living in sediments. Unfortunately, there is not a single method able to quantify all the parameters related to sediment pollution, such as heavy metals and organic micropollutants concentration, in order to obtain a diagnostic on their impact on biota. Thus, the use of several complementary methods rather than a single one is becoming a standard practice to obtain independent information of the degree and possible impacts of sediment contamination.

The integrated application of different sediment assessment methods can be proposed in a diagnosis and/or remediation project if the whole cost of the analysis is reasonable. In this sense, less expensive analytical protocols are required to obtain valuable information. However, in recent studies [4] soil and sediment pollution characteristics have been predicted through the modelization of IR data in both, middle [5] and near [6] domains and mineral phases have been identified by Raman spectroscopy [7], differentiating those of natural origin from those of anthropogenic sources. Raman spectroscopy has been also used to evaluate the interaction between calcium, magnesium and arsenic in estuarine media [8].

In spite of the interesting results obtained for trace element prediction in sediments by using vibrational spectroscopic techniques it is clear that vibrational spectrum of mineral elements in sediment samples is quite poor and prediction capabilities of partial least squares (PLS) models are based in small changes in the signals due to the interaction between the organic matter and the pollutants [5,6].

Destructive methods as inductively coupled plasma/optical emission spectroscopy (ICP-OES), and especially, inductively coupled plasma/mass spectrometry (ICP-MS) are widely used for multi elemental analysis of sediment samples [9,10] but are costly and too much time is required to perform the analysis. On the other hand, from a green analytical point of view, X-ray fluorescence (XRF) provides a reagent-free environmentally friendly and non-destructive technique, which permits to determine the mineral content of samples without any complex sample pre-treatment, thus minimizing laboratory space, resources and safety implications, also avoiding the use of chemical reagents. Furthermore, XRF presents the possibility of making in-situ analysis and could be considered a green technique [11]. The secondary XR fluorescence spectrum identifies the elements present in the sample and its intensity is proportional to their concentration. The disadvantage of the XRF technique is that the detection limits (typically mg·Kg^{-1}) are higher than those obtained by using ICP-OES or ICP-MS for dissolved trace elements and that the quantitative processing of the spectral data using external calibration is time consuming, as compared with other instrumental techniques in order to avoid matrix sample interferences, and requires skilled operators. So, XRF is normally used for semi-quantitative and quantitative multi-elemental analysis of mineral phases in pigments, rocks, sediments, soils and contaminated lands [12, 13].

A large variety of multivariate methods are used in combination with XRF results. Orthogonal multivariate regression models are those most employed for quantitative analysis, as well as principal components regression (PCR) and partial least square (PLS) [14]. PLS combines spectral and analytical information of a number of samples to built predictive models by establishing a direct correlation between spectra of samples and properties of interest (generally concentration values) of the species contained in them. All these implementations to guarantee the accuracy in quantifying trace element concentrations have opened the possibility to use XRF as a rapid and low cost technique to obtain basic information of total concentration of metals in the assessment of potentially polluted soils and sediments [15].

The purpose of the present paper has been the development of a new analytical methodology, based on the combination of energy-dispersive X-ray fluorescence (μ-EDXRF) measurements and chemometric data treatment to quantify the amount of several elements in a unique step through the evaluation of selective regions from the whole spectrum. Developed method has been tested for the determination of minor and trace elements in sediment samples, corresponding to eight sampling locations and four sampling campaigns per year (during 2005-2008), of the estuary of the Nerbioi-Ibaizabal River (Bay of Biscay, Basque Country). Reference values of trace elements concentration corresponding to those samples used for both building and evaluating PLS models were obtained by ICP/MS after acid leaching of the sediment.

2. Experimental

2.1. Sampling, sample preparation and analysis

A total amount of 116 sediment samples were collected from eight sampling locations in the estuary of the Nerbioi-Ibaizabal River (Metropolitan Bilbao, Basque Country, north of Spain), located on the continental shelf of the Cantabrian coastline in the south-eastern part of the Bay of Biscay [16] (see Figure 1).

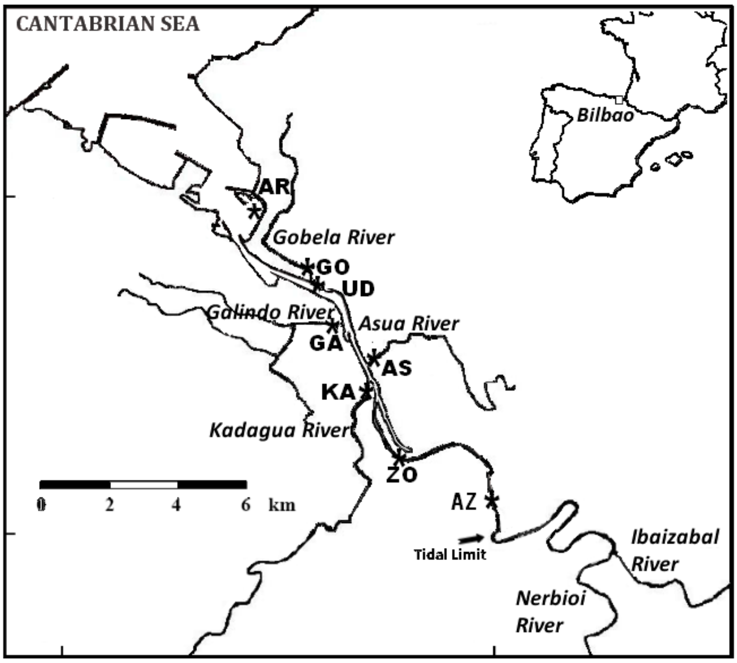

Figure 1. Nerbioi-Ibaizabal estuary. Note: sampling sites are indicated with star marks: Alde Zaharra (*AZ*), Zorroza (*ZO*), Kadagua (*KA*), Asua (*AS*), Galindo (*GA*), Udondo (*UD*), Gobela (*GO*) and Arriluze (*AR*).

Sediments were sampled approximately every three months (15 sampling campaigns from January 2005 to October 2008), using plastic sampling materials and latex gloves to avoid sample contamination with metals. Approximately 500 g of surface sediment samples (~2 cm depth) were collected at low tide, from eight well identified stations strategically distributed along the estuary. Samples were set inside cleaned plastic bags and transported to the laboratory in portable cooler boxes at 4 ºC to reduce the effects of microbiological activity.

In the laboratory, sediment samples were frozen at -20 ºC and then lyophilized at 150 mTorr and -52 ºC in a Cryodos apparatus (Telstar, Terrassa, Spain) for 48 h. Finally, samples were sieved at 63 µm and kept in the refrigerator at 4 ºC until analysis.

XRF were obtained using the portable ArtTAX µ-EDXRF equipment by Röntec (nowadays Bruker AXS) equipped with a molybdenum anode working at a maximum voltage of 50 kV and a maximum current of 0.7 mA. The X-rays are collimated by a tantalum collimator with a diameter of 1.5 mm. The equipment also includes a CCD camera which allows to obtain an image of the sample analyzed (see inset of Figure 2) and a motor-driven XYZ positioning unit to focus on different parts of the samples controlled by the computer. 200 mg of each homogenized sediment sample compacted under a total pressure of 10 tons, thus providing sample pellets with 10 mm diameter and 1 mm width. Independent triplicate spectra were obtained for each pellet. All the measurements were performed with an exposition time of 1000 s at a voltage of 50 kV and a current of 0.6 mA. For the identification and quantification of elements with a low atomic number a helium flow (99.999 %, Air liquid, Spain) was used. All spectra processing and manipulation was carried out by using ARTAX 4.9.13.2 program from Bruker AXS.

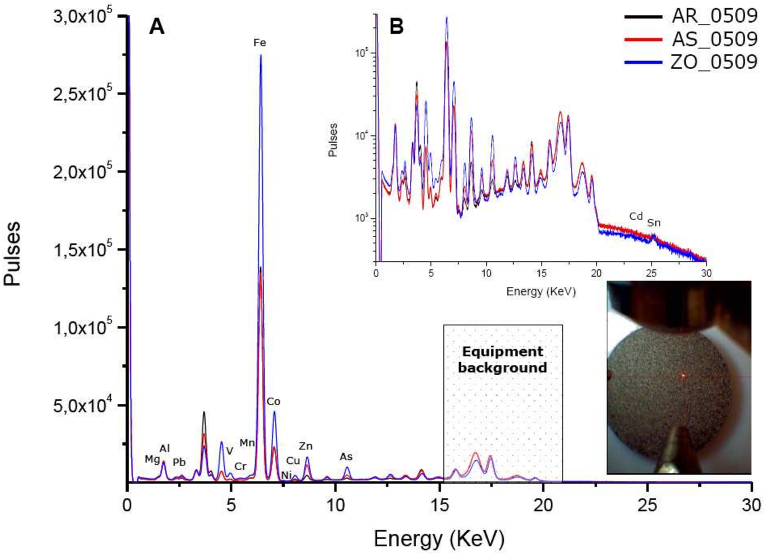

Figure 2. Micro-XRF spectra acquired from the surface of samples corresponding to different sampling points (ARriluze, ASua and ZOrrotza) obtained in 2005 September: A) lineal and B) logarithmic scale for intensity values. Inset: Photography showing sample pellet measuring point to obtain XRF spectra.

The extractable concentration of Al, As, Cd, Co, Cr, Cu, Fe, Mg, Mn, Ni, Pb, Sn, V and Zn in the sediment samples were determined by ICP-MS after acid (HNO_3 and HCl mixtures) digestion accelerated with focused ultrasound energy as described elsewhere [17]. During the extraction step samples were sonicated with a HD 2070 Sonopuls Ultrasonic Homogenizer (Bandelin, Germany) equipped with a GM 2070 generator (70W, 20 KHz), an UW 2070 ultrasonic converter, a SH 70 GQ horn and GS 6 glass probe (6 mm). The analysis of the extracts was carried out by inductively coupled plasma/mass spectrometry (ICP-MS) using an Elan 9000 ICP-MS spectrometer from Perkin-Elmer (Ontario, Canada), equipped with a Ryton cross-flow nebulizer, a Scott-type double pass spray chamber and standard nickel cones. Preparation of calibrantions and sample analysis was done inside a clean room (class 100). The nitric acid (69%, Tracepur) and hydrochloric acid (36%, Tracepur) used in the extraction step were purchased from Merck (Darmstadt, Germany). Milli-Q (Millipore, Billerica, MA, USA) quality water with a conductivity lower than 0.05 $\mu S \cdot cm^{-1}$ was used to prepare sample and standard solutions. Multielemental standard solutions of the analytes were prepared by weight in 1% HNO_3 from individual commercial stock solutions (Specpure from Alfa Aesar, War Hill, MA, USA) of Al, As, Cd, Co, Cr, Cu, Fe, Mg, Mn, Ni, Pb, Sn, V and Zn (1000 $mg \cdot L^{-1}$ in 5% HNO_3). Be^9, Sc^{45}, In^{115} and Bi^{209}, also from Specpure stock standard solutions (1000 $mg \cdot L^{-1}$ in 5 % HNO_3), were added to blank, standard and sample solutions as internal standard to yield a 10 $\mu g \cdot L^{-1}$ concentration level. 99.999 % argon (Praxair, Spain) was used as plasmogen and carrier gas in the ICP-MS. The certified reference material NIST-SRM 1646a (estuarine sediment from the National Institute of Standards and Technology, Gaithersburg, USA) was used to validate the accuracy of the ICP-MS analytical procedure.

These determined extractable concentrations values were used as reference data for building the partial least squares (PLS) multivariate models as well as for the subsequent evaluation of their predictive capabilities.

2.2. Chemometric data treatment

Hierarchical cluster analyses (HCA) of XRF-spectra as well as PLS models for mineral elements determination were developed by using OPUS program Version 4.1 from Bruker Gmbh (Bremen, Germany). HCA is an exploratory data tool used for describing a system of organized observations through their classification into groups of samples sharing properties in common (see reference [18] for additional information on HCA). In this study HCA was used to evaluate the spectra differences between samples considered in order to select an appropriate subset of samples for building the PLS prediction models [19]. Previous to HCA a vector normalization spectral pre-treatment was applied. For PLS modeling XRF-spectra were mean-centered and after that different spectral pretreatments were applied to obtain calibration models with a high predictive capability for each element considered in this study. The root mean square error of calibration (RMSEC), the root mean square error of prediction (RMSEP), the coefficient of multiple correlation (r) and the mean of the differences between the predicted spectroscopic PLS values and ICP-MS reference data (d_{x-y}), together with the pooled standard deviation for validation samples (strip) and the residual predictive deviation (RPD), ratio of validation data set standard deviation to RMSEP (SDR) were employed as quality indicators of the different models assayed [20].

3. Results and discussion

3.1. Reference data from trace elements

Metal concentrations in sediment samples used in the present work were obtained by the reference ICP-MS procedure [17] and correspond to the mean of 4 sample replicates, being precission values (reproducibility between days) comprised between 1.1% for Pb and 4.7% for Mn. Element contents in sediment samples considered in this study ranged between 900 and 15500 $\mu g \cdot g^{-1}$ for Al, from 0.6 to 220 $\mu g \cdot g^{-1}$ for As, from non detected to 18 $\mu g \cdot g^{-1}$ for Cd, between 0.3 and 32 $\mu g \cdot g^{-1}$ for Co, from 5 to 175 $\mu g \cdot g^{-1}$ for Cr, between 15 and 575 $\mu g \cdot g^{-1}$ for Cu, from 5200 to 36720 $\mu g \cdot g^{-1}$ for Fe, between 330 and 8340 $\mu g \cdot g^{-1}$ for Mg, from 65 to 935 $\mu g \cdot g^{-1}$ for Mn, between 3.3 and 320 $\mu g \cdot g^{-1}$ for Ni, from 21 to 445 $\mu g \cdot g^{-1}$ for Pb, from non detected to 68 $\mu g \cdot g^{-1}$ and 37 $\mu g \cdot g^{-1}$ for Sn and V, respectively and between 40 and 2060 $\mu g \cdot g^{-1}$ for Zn.

3.2. X-ray fluorescence spectra of sediment samples

Figure 2 shows the characteristic XRF spectra of three samples taken from different points of the estuary during different sampling campaigns. As it can be seen the spectra contain characteristic peaks of the analytes of interest and non-explicit information, also suitable to be modeled as a function of the concentration of trace elements in samples of the same type previously characterized by a specific and well validated methodology. Additionally, the micro X-ray spectra obtained from the surface of the sediment pellets include the Compton and Rayleigh scattering lines of Mo and Zr, the source and a component of the detector and the collimator which are employed to establish the equipment background.

All collected spectra were classified using the Euclidean distance with Ward linkage upon considering the voltage range between 0.5 and 28 KeV on the raw spectra, being considered the spectral regions between 28 KeV and 50 KeV as useless and uninformative in order to determine the trace element concentration in the Nerbioi-Ibaizabal estuary.

Figure 3 depicts dendrographic classification obtained from sediment sample spectra and as seen, 11 clusters are well defined for a cut-off value of $0.7x10^6$.

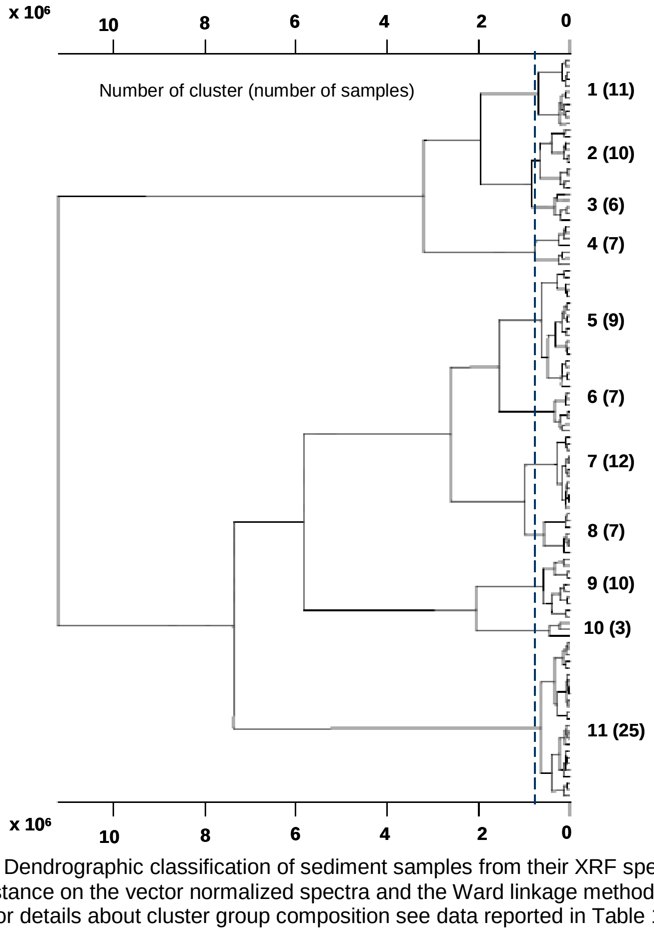

Figure 3. Dendrographic classification of sediment samples from their XRF spectra. The Euclidean distance on the vector normalized spectra and the Ward linkage methods were used. For details about cluster group composition see data reported in Table 1.

Samples grouped in each cluster joint together the averaged value for the concentration of each element, companied by its standard deviation, for overall sediment samples contained in each group are detailed in Table 1.

A relative correlation between clusters structure and regions across the estuary can be established: i) clusters 1 to 4 and clusters 9 and 10 contain sample spectra from Galindo (*GA*), Gobela (*GO*) and Udondo (*UD*) locations (that correspond to the most polluted sediment sites in the estuary [16]), being cluster 10 totally integrated by the spectra of *GA* sediments sampled in 2008 and cluster 4 by spectra of *GA* sediments taken in 2005 and 2007; ii) clusters 5 to 8 and cluster 11 contain sample spectra from sediments collected in Arriluze (*AR*), Asua (*AS*), Alde Zaharra (*AZ*), Kadagua (*KA*) and Zorroza (*ZO*) locations (the less polluted sites in the estuary), being cluster 8 integrated by the main part of samples collected in *KA* from 2005 to 2007.

In fact, clustering seems to be mainly correlated with the concentration of elements exhibiting the most sensitive X-ray fluorescence signals. However there is not a simple correlation between trace element concentration and sample clustering, being thus the classification dependent on the matrix sediment additionally than on the trace element composition. So, in the present study HCA was used to evaluate the spectra differences between samples considered in order to select an appropriate subset of samples for building the PLS prediction models (see references [6 and 19] for additional details on the use of HCA for properly selecting a representative calibration set for modeling and also for verifying the nature of new samples previously to be predicted). Table 2 summarizes the descriptive statistics of the calibration set established from 34 samples selected from the 11 clusters and validation independent set, composed by the 82 available samples not used for calibration. As it can be seen trace element concentration of validation samples are included in the range of the calibration set, thus avoiding the extrapolation of data. Samples employed for calibration were randomly selected including samples of all cluster, taken from different sampling locations till to have the rounded valued of the root square of the total number of samples included in each cluster.

Table 1. Characteristics of the Nerbioi-Ibaizabal estuarine sediment samples classified into clusters after dendrographic treatment of XRF data.

Cluster number (number of samples in each clustetr)					
Element	**1 (11)**	**2 (10)**	**3 (5)**	**4 (7)**	**5 (19)**
Al	4870 ± 1254	4799 ± 461	5680 ± 2897	5061 ± 1946	3808 ± 2326
As	85 ± 47	87 ± 70	145 ± 67	61 ± 58	15 ± 14
Cd	5 ± 3	7 ± 3	7 ± 4	4 ± 3	2 ± 3
Co	7 ± 2	6.2 ± 1.0	7.7 ± 1.1	10 ± 4	7 ± 3
Cr	44 ± 12	54 ± 15	62 ± 16	65 ± 12	42 ± 28
Cu	278 ± 158	301 ± 126	325 ± 95	194 ± 62	59 ± 38
Fe	18167 ± 4694	22434 ± 7423	24599 ± 5615	22334 ± 6745	10776 ± 2627
Mg	3085 ± 881	3128 ± 660	2203 ± 1226	2250 ± 661	2841 ± 1795
Mn	348 ± 199	500 ± 312	328 ± 231	473 ± 151	277 ± 125
Ni	22 ± 8	29 ± 11	21 ± 6	69 ± 39	26 ± 15
Pb	235 ± 72	240 ± 61	307 ± 72	259 ± 72	93 ± 75
Sn	26 ± 7	31 ± 10	38 ± 12	32 ± 10	10 ± 5
V	22 ± 8	19 ± 12	29 ± 7	13 ± 9	6 ± 6
Zn	829 ± 353	979 ± 309	1069 ± 290	872 ± 248	346 ± 213
Samples corresponding to each cluster					
	AR_05-05 GA_06-10 GO_05-01 GO_06-10 GO_07-01 GO_07-04 GO_07-10 UD_06-10 UD_07-01 UD_07-04 UD_07-10	GA_05-12 GA_06-03 GA_06-07 GO_05-12 GO_06-03 GO_06-07 GO_08-04 UD_05-12 UD_06-03 UD_06-07	GO_05-05 GO_07-07 UD_05-05 UD_05-09 UD_07-07	GA_05-01 GA_05-05 GA_07-01 GA_07-04 GA_07-07 GA_07-10 UD_05-01	AR_05-09 AR_07-07 AR_08-04 AR_08-07 AS_05-09 AS_08-01 AS_08-04 AS_08-07 AZ_05-09 AZ_07-04 AZ_07-10 AZ_08-01 AZ_08-04 KA_08-01 KA_08-04 KA_08-07 ZO_05-09 ZO_08-01 ZO_08-04

Notes: Samples are identified with two letters (corresponding to the sampling point) and 4 numbers (the first two numbers for the year and the last ones corresponding to the month of sampling).
All trace element concentration values correspond to the averaged value from the concentration of all samples contained in the cluster, expressed in µg·g^{-1} units. ± s refers to the standard deviation of the average value.

Table 1 (continued). Characteristics of the Nerbioi-Ibaizabal estuarine sediment samples classified into clusters after dendrographic treatment of XRF data

Element	**Cluster (number of samples)**					
	6 (7)	**7 (12)**	**8 (7)**	**9 (10)**	**10 (3)**	**11 (25)**
Al	1816 ± 1590	5550 ± 3936	4226 ± 3075	3877 ± 2140	4107 ± 1770	4434 ± 1790
As	11 ± 11	20 ± 15	3.5 ± 1.8	79 ± 39	45 ± 16	17 ± 14
Cd	0.9 ± 0.8	2.0 ± 1.9	0.01 ± 0.03	4 ± 2	3.6 ± 0.6	2 ± 4
Co	9.3 ± 1.0	5 ±3	3.4 ± 1.8	9 ± 2	19 ± 11	6 ± 2
Cr	29 ± 10	33 ± 10	8 ± 3	41 ± 7	89 ± 75	36 ± 19
Cu	49 ± 22	68 ± 31	39 ± 53	227 ± 145	187 ± 88	81 ± 56
Fe	8379 ± 1601	10310 ± 2752	6626 ± 1074	17954 ± 6627	23916 ± 6825	10562 ± 1928
Mg	5739 ± 1841	2681 ± 2550	1362 ± 673	4522 ± 1866	4645 ± 2573	2360 ± 1465
Mn	227 ± 89	339 ± 241	249 ± 102	318 ± 151	574 ± 148	241 ± 146
Ni	34 ± 20	18 ± 4	6 ± 2	28 ± 17	164 ± 136	21 ± 7
Pb	92 ± 82	120 ± 79	44 ± 19	223 ± 78	321 ± 89	119 ± 102
Sn	8 ± 4	9 ± 6	3 ± 3	25 ± 8	40 ± 24	12 ± 6
V	6 ± 4	5 ± 6	2 ± 3	20 ± 4	11.0 ± 1.7	5 ± 4
Zn	280 ± 139	583 ± 426	190 ± 244	765 ± 178	1009 ± 195	500 ± 457

Samples corresponding to each cluster

	AR_08-10 AS_08-10 AZ_08-07 AZ_08-10 KA_08-10 ZO_08-07 ZO_08-10	AR_05-12 AR_06-03 AR_06-07 AS_05-12 AS_06-03 AS_06-07 AS_07-07 AZ_05-12 AZ_06-03 ZO_05-12 ZO_06-03 ZO_06-07	KA_05-01 KA_05-05 KA_05-12 KA_06-03 KA_06-07 KA_07-07 KA_07-10	AR_08-01 GA_08-07 GO_05-09 GO_08-01 GO_08-07 GO 08-10 UD_08-01 UD_08-04 UD_08-07 UD_08-10	GA_08-01 GA_08-04 GA_08-10	AR_06-10 AR_07-01 AR_07-04 AR_07-10 AS_05-01 AS_05-05 AS_06-10 AS_07-01 AS_07-04 AS_07-10 AZ_05-01 AZ_06-07 AZ_06-10 AZ_07-01 AZ_07-07 KA_06-10 KA_07-01 KA_07-04 ZO_05-01 ZO_05-05 ZO_06-10 ZO_07-01 ZO_07-04 ZO_07-07 ZO_07-10

Notes: Samples are identified with two letters (corresponding to the sampling point) and 4 numbers (the first two numbers for the year and the last ones corresponding to the month of sampling).

All trace element concentration values correspond to the averaged value from the concentration of all samples contained in the cluster, expressed in $\mu g \cdot g^{-1}$ units. ± s refers to the standard deviation of the average value.

Table 2. Descriptive statistics for calibration and validation datasets used for PLS-XRF based prediction of trace elements in estuarine sediment samples and figures of merit of reference method based on ICP-MS measurements [17].

Element	**Sets (number of samples)**		**Reference ICP-MS method**	
	Calibration (34)	**Validation (82)**	**Repeatability (%)**	**LD (μg·g^{-1})**
[Al]	4903 ± 3236	4169 ± 1817	2.9	18
[As]	50 ± 61	40 ± 45	3.7	0.9
[Cd]	4 ± 4	3 ± 3	2.1	1.0
[Co]	8 ± 3	7 ± 4	4.0	0.2
[Cr]	42 ± 22	42 ± 25	2.5	0.5
[Cu]	154 ± 126	137 ± 131	1.7	23
[Fe]	15483 ± 8146	13731 ± 6395	4.2	12
[Mg]	3425 ± 2121	2799 ± 1696	3.3	6.0
[Mn]	372 ± 220	301 ± 182	4.7	0.9
[Ni]	27 ± 24	30 ± 37	2.6	4.0
[Pb]	180 ± 126	153 ± 101	1.1	1.0
[Sn]	19 ± 15	17 ± 12	2.2	1.9
[V]	13 ± 11	10 ± 9	3.5	0.3
[Zn]	679 ± 479	577 ± 375	2.0	4.0

Note: All values are expressed in μg·g^{-1} units. Averaged ± its corresponding standard deviation concentration values of each trace metal calculated from the overall samples included in each set. Values indicated between brackets correspond with minimum and maximum concentration levels found for samples considered for each set. Repeatability values were obtained from 4 replicates. LD data were calculated from the standard deviation of 8 measurements of a blank.

3.3. Partial least square modelization of XRF data for trace metals prediction

Partial least square (PLS) multivariate models were built from the selected calibration set of XRF spectra of sediment samples.

Several spectral regions in the voltage domain [21] as well as different spectra treatments [22] were tested. Models were compared in terms of RMSEP values of trace metal content for samples not used for calibration in order to evaluate the model prediction capabilities. However, as a consequence of the high volume of results generated, only the best ones have been summarized in this paper. Fitting features as well as prediction capabilities of models obtained for each trace element are summarized in Table 3. Spectral region was optimized for each element under consideration by splitting the overall selected spectral range (0.5 keV – 28 keV) in 10 equally large regions and successively calculating combinations of them. In the case of Fe and Cr determination a refined selection of the spectral interval was made by splitting spectral ranges comprised between 5 - 9 KeV and 5 – 7 KeV, respectively. As it can be seen, from one to four combined spectral regions may be used to obtain the best prediction capability in each case.

Additionally, from none to different spectral treatments such as first (FD) or second (SD) order derivative, multiplicative scattering correction (MSC), constant offset elimination (COE), vector normalization (VN), straight line subtraction (SLS) and min-max normalization (MMN) as well as a combination between some of them (FD+SLS) were also required in some cases. Regarding the number of PLS factors they were selected to obtain the minimum RMSECV to have a good predictive capability. This parameter ranged from a minimum value of 1 (for Sn) to a maximum one of 10 (for As), being this parameter a descriptive index on the complexity of the relationship between XRF spectra and element concentration.

In order to evaluate the sensitivity of the proposed PLS-XRF method the limit of detection values for each element were established from 10 measurements of a blank spectra of the sample holder and air. LD obtained varied from 0.4 $\mu g \cdot g^{-1}$ for Cd to 70 $\mu g \cdot g^{-1}$ for Al and, in some cases, were of the same order than those provided by the reference method applied to sediment analysis (see Table 2).

As an example, Figure 4 shows the comparison between the calibration and validation sets for arsenic (As) and tin (Sn), as elements with low concentration in the studied system, and copper (Cu) and lead (Pb), as elements with relative high concentrations in all the samples. As it can be appreciated, fitting for calibration and validation results are coincident within the confidence interval of the respective slopes, thus indicating that there are not significant differences between both, calibration and validation data sets.

Table 3. Modelling parameters and prediction capabilities of PLS-XRF models developed for the determination of trace elements in estuarine sediment samples from the Nerbioi-Ibaizabal estuary.

Element	Spectral range (KeV)	Spectral pre-processing
Al	28.0 – 25.3 / 22.5 – 19.8 / 3.3 – 0.5	None
As	28.0 – 19.8 / 17.0 – 8.7	Vector normalization
Cd	25.3 – 22.5 / 14.3 – 8.7 / 3.3 – 0.5	Vector normalization
Co	28.0 – 22.5 / 19.8 – 17.0 / 11.5 – 6.0	Second derivative
Cr	6.8 – 6.6 / 6.4 – 6.2 / 5.8 – 5.2	Straight line subtraction
Cu	22.5 – 17.0 / 8.8. – 6.0	Straight line subtraction
Fe	9.0 – 8.6 / 8.2 – 7.8 / 7.4 – 7.0	Min-Max Normalization
Mg	25.3 – 19.8 / 14.3 – 0.5	First derivative
Mn	6.0 – 0.5	First derivative + Straight line subtraction
Ni	8.8 – 6.0	None
Pb	14.3 – 11.5	Straight line subtraction
Sn	25.3 – 22.5 / 19.8 – 17.0 / 14.3 – 8.7 / 3.3 – 0.5	Constant offset elimination
V	25.3 – 19.8 / 14.3 – 8.7	Multiplicative scattering correction
Zn	28.0 – 22.5 / 17.0 – 8.7 / 3.3 – 0.5	None

Table 3 (continued). Modelling parameters and prediction capabilities of PLS-XRF models developed for the determination of trace elements in estuarine sediment samples from the Nerbioi-Ibaizabal estuary.

Element	F	r	RMSEC ($\mu g \cdot g^{-1}$)	RMSEP ($\mu g \cdot g^{-1}$)	RRMSEP (%)	$d_{(x-y)}$ ($\mu g \cdot g^{-1}$)	s_{trip} ($\mu g \cdot g^{-1}$)	*RPD*
Al	2	0.61	2601	1708	41	472	223	1.1
As	10	0.97	6	11	29	-0.19	9	3.9
Cd	4	0.84	1.9	1.7	62	0.3	0.3	1.8
Co	7	0.72	2.4	2.8	40	-0.16	0.5	1.4
Cr	7	0.76	10	16	39	1.6	8	1.5
Cu	9	0.95	42	44	32	10	23	3.0
Fe	8	0.79	4085	4240	31	1505	1365	1.5
Mg	9	0.85	961	910	32	9	257	1.9
Mn	8	0.86	80	94	31	13	29	1.9
Ni	9	0.75	7	26	87	-5	7	1.4
Pb	7	0.95	21	31	21	1.7	16	3.2
Sn	1	0.88	6	6	33	-0.13	1.5	2.1
V	4	0.92	5	4	35	0.12	1.1	2.6
Zn	7	0.78	127	236	41	3	35	1.6

Notes: F is the number of PLS factors employed to build the model, r is the correlation coefficient, RMSEC and RMSEP are the root mean square error of calibration and prediction, respectively. RRMSEP is the RMSEP divided by the mean valu of trace element in the validation dataset. s_{trip} is the standard deviation of three replicates. $d_{(x-y)}$ is the mean difference between predicted and reference trace element content value. RPD is the residual predictive deviation.

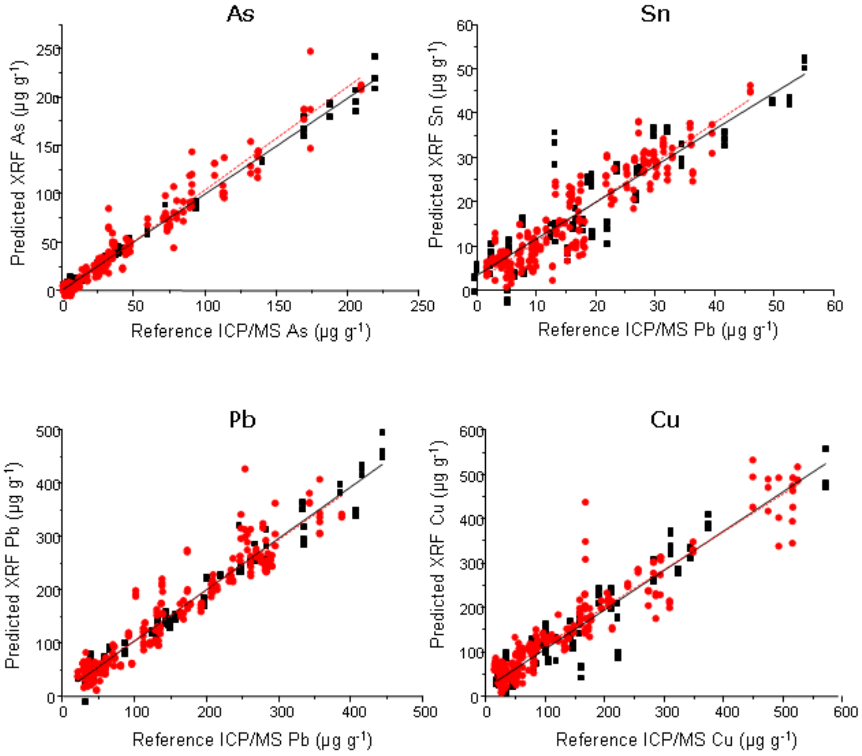

Figure 4. Regression between PLS-XRF predicted data and reference concentration values obtained by ICP-MS for the determination of As, Cu, Pb and Sn in Nerbioi-Ibaizabal estuary sediment samples. Concentration values are expressed in μg·g^{-1}. ■: calibration samples and ●: validation samples.

The high fluctuation of concentration values is due to the seasonal trends observed in this estuary (minimum content in winter and maximum in summer) for the whole set of considered elements [6].

XRF prediction capabilities provided better results than those achieved in a previous study using vibrational spectroscopy [6]. XRF models provided, as a trend, the highest RPD values, RRMSEP values ranging between 21% and 41%, with few exceptions given for Cd (62%) and Ni (87%), and r values spanned from 0.72 to 0.97 (avoiding Al results) which are also better than those obtained by vibrational models. Nevertheless, optimal number of factors to be considered in the vibrational PLS models were, in general, lower than those determined for XRF-PLS models.

4. Conclusions

It has been demonstrated, from results obtained in the present study, that the concentration of several trace elements as As, Cd, Cr, Cu, Fe, Mg, Ni, Sn, V and Zn in estuarine sediments from the Nerbioi-Ibaizabal River (Metropolitan Bilbao, Bay of Biscay, Basque Country) could be properly modelized (exception given for Al, Co, and Ni exhibiting RPD values lower than 1.5) through the combined use of XRF spectra and PLS treatment. It results in a green analytical tool for environmental studies suitable to extract concentration data at trace levels from spectra of untreated samples just doing an appropriate calibration, with samples previously analyzed by a reference procedure, and considering some spectral pre-processing strategies. The fact that the XRF spectra contain both, intrinsic and extrinsic information on the mineral composition of samples is clearly the reason to obtain more accurate results by XRF than those previously found from vibrational spectrometry and evidences the capability of PLS based analytical procedures for processing simultaneously information regarding to different chemical compounds and obtained from different analytical signals.

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Conclusions

Take its pulse and blood pressure and check whether it has a temperature is what we have done to the river estuary. The time has arrived to diagnose the patient's overall health.

As has been mentioned on repeated occasions throughout this thesis, water is the natural compartment that best reflects the current situation of the river estuary, amongst other reasons, because it contains the fraction of the pollutant that is actually bioavailable. However, water analysis would make no sense if sediment status were not taken into account. Therefore, a simultaneous study of both is essential.

From the results obtained, it can be said without doubt that historic pollution derived from human activity still has a notable presence in sediment. According to the geoaccumulation indexes (I_{geo}) calculated, measured concentrations are significantly higher than the background values estimated for the area. In view of the spatial distribution of most metals, historic sediment pollution can be said to be the result of **point source pollution**. Currently, as shown by the results of the extensive sampling, trace elements accumulate in particular in sediments from the Gobela and Galindo river areas and from a number of wharfsides (Axpe, Udondo and Deustu). Apart from this historic pollution, current sources of **diffuse (nonpoint)** and **point source** metal pollution determine the present state of pollution in the river estuary in both water and sediment (see Figure. 1).

In the six year period studied, overall, concentration values for trace elements in water rose, and this trend is more noticeable in samples collected at high tide and close to the river bed, that is in water that comes through the estuary mouth by effect of the tide. It may be that some activity (or activities) in the Abra area is acting as a **point source of pollution**, spreading pollution throughout the river estuary at each high tide and/or changing some physicochemical property of the water (pH or percentage of dissolved oxygen, for example). The work being carried out to build the super-port or shipyards whose activity is based in the Abra Bay may be responsible for this trend. In any case, the observed upward trend is not the same in all metals; certain elements (Cd and Zn, for example) have shown a sharp rise since April 2009. The pH plot over time shows a similar trend. In connection with this, there is another possible point source of pollution that could affect metal content values in water, which is the estuary sediment itself.

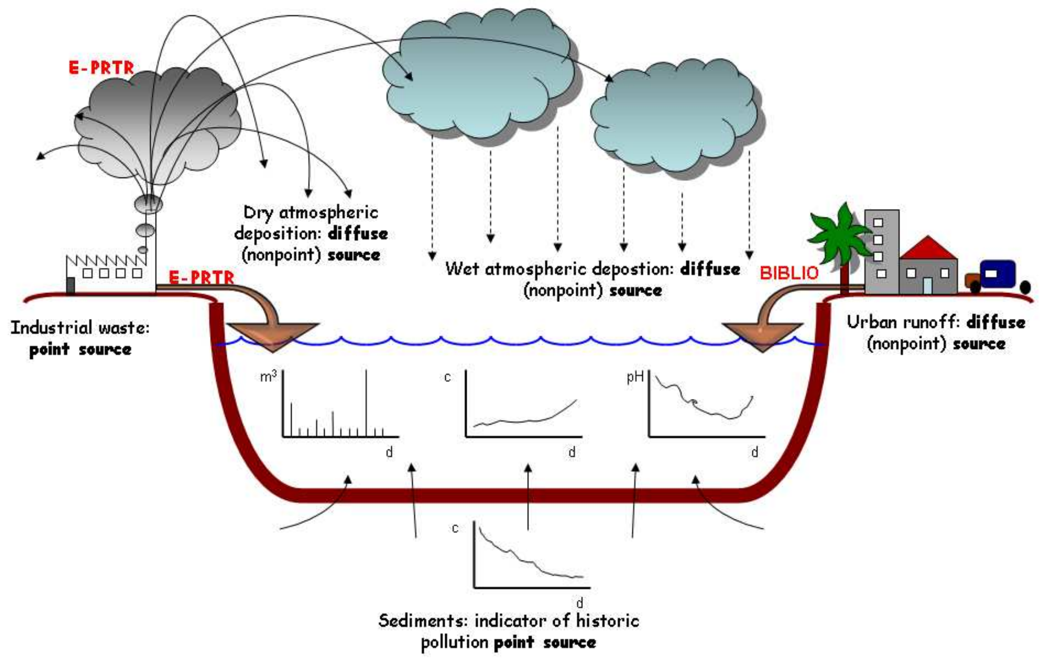

Figure 1. Overall scheme of sources of pollution that reaches the river estuary.

Metal content in this compartment has fallen over time, particularly at points that show high concentrations of metals (*UD*, *GO* and *GA*). In sediment gathered at points of low metal content in the estuary (*AZ*, *ZO* and *KA*), in contrast, some metals show a positive trend. However, between 2008 and 2010 this trend was far less pronounced. Based on this information our research group[4] proposed that metal pollution in sediment was redistributing itself. Whilst this theory has not been completely abandoned, it has been relegated to a secondary position, and the idea that what is lost from the sediment is accumulating in the water is gathering strength.

The negative trend displayed by metal concentrations in water over time, may be linked to the downward trend observed in the pH of the water. Indeed, below the usual pH of the estuary, the most available species that

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[4] Fdez-Ortiz de Vallejuelo S. 2008. *Diagnóstico, evolución y predicción de la concentración metálica en el estuario del río Nerbioi-Ibaizabal*.Doctoral Thesis. University of the Basque Country, Bilbo.

trace elements have in sediment (hydroxides, to be precise) can dissolve, releasing metals into the water. An in-depth study of the variability of the pH and a number of metal concentrations over time shows partial upward trends after heavy rainfall within an overall downward trend. The trend that starts in April 2009 is particularly notable and lasts until the last campaign. After heavy rainfall, the force of the total quantity of water flowing into the river estuary (both from tributaries and from urban runoff) remobilises the upper layers of sediment, facilitating the mixing process of water and sediment. The effects of this remobilisation are probably not immediate and have the most impact in the following campaign. Bearing in mind the tremendous downpours in the Bilbao area in January 2009 (causing a 180 $m^3 \cdot s^{-1}$ water flow rate) the after effects were expected to be particularly marked. These expectations were borne out by the dramatic rise in pH and in the concentration values of certain metals in the water.

Apart from the pollution sources mentioned so far (bottom salt water coming from the Abra Bay itself and sediment), we have identified other possible point sources of pollution. Observation of the results of analyses of water samples taken at low tide and from the surface (river water), enables the path of each trace element into the estuary to be determined. In this process the information available in the EPRTR (*European Pollutant Release and Transfer Register*) is of great help. The register shows that at present there are still factories that discharge waste water directly into the river. Although all the tributaries suffer from pollution, Galindo river, is by far the most polluted, particularly in terms of Zn and Ni content (and most probably Co too). The results of sediment gathered at Galindo confirm the Galindo water treatment plant as a source of pollution. Taking into account the volume of waste water that is treated there, what is happening is understandable, but looking towards the future, finding a way to turn the situation around should be a priority.

Apart from point source pollution, there are also sources of **diffuse pollution** in the river estuary. This is pollution which is spread throughout the whole estuary, which has a similar impact at all points. It can have different sources, amongst others i) dry or wet atmospheric deposition (industrial metal pollution of the atmosphere) and ii) urban runoff, the small bodies of water that form on roads and in built-up areas in times of heavy rain, which finally reach waterways through the sewer system. Although the measurements taken in this work do not enable an estimation of the quantity of trace elements that reach the river estuary from diffuse sources, these source can be considered to contribute significantly to pollution, as the information in the E-PRTR database on discharges into the atmosphere shows. Urban runoff is also suspected to be an important contributor of pollution. The possibility of sending runoff to a water treatment plant after some simple pretreatment has been looked into in recent times[5].

The pysicochemical differences between water samples taken at high tide from the bottom, on the one hand, and at low tide from the surface on the other, show that the Nerbioi-Ibaizabal River estuary is clearly stratified. However, in each layer the horizontal variability is different. Whilst the difference between points in bottom water at high tide is small, there is a distinct lack of homogeneity in surface water at low tide. In sediment too, there are very clear differences between sampling points. There are still highly polluted areas, particularly in the middle part of the estuary. Using the results of the extensive sampling, the area between the Asua tributary and Lamiako dock (including the most inner part of the Deustu Canal), and the outer area (the Abra Bay area) have been defined as the most highly polluted environments.

However, although the concentration values of trace elements found in the sediment in these areas far exceed the estimated natural background values, from a toxicological point of view, and based on calculated mERMq values, they do not pose an immediate threat.

Therefore, with the implementation of the WFD in mind, it would seem that, in terms of metal pollution, the situation of the Nerbio-Ibaizabal River estuary has improved. However, in order to confirm this conclusion, mass balances should be performed, taking into account not only water and sediment but also suspended particles, since they play an important part in the system. This compartment is fundamental to a study of the natural cycle

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[5] Hydrodynamic separator, helping to protect water in the Nerbio-Ibaizabal river estuary. 2010, Urgarbi. http://issuu.com/urgarbi/docs/cgz?viewMode=presentation&mode=embed

of metals in river estuaries as, depending on physico-chemical conditions, they work in favour of a natural cleaning process in the river estuary (when particles loaded with metals are flushed out to sea) or against it (when particles loaded with metals accumulate forming the upper layers of sediment).

In order to reach all of the conclusions above, however, it has been necessary to perform lengthy analytical procedures that use hazardous substances. For that reason alternative methods for determining metal content in sediment have been developed that are in line with the principles of green chemistry. It is clear that the alternative methods developed to date are appropriate. The calibration model based on X-ray fluorescence has proved to be more efficient than the model based on infrared spectroscopy. This is most likely a direct result of the characteristics of the method: XRF provides information about elemental composition, whereas infrared spectroscopy provides information about molecular composition. Both require less effort in experiments than classical approaches, as they do not involve any treatment of dry samples (extraction, dilution…). They do have some drawbacks, however. The process of calculating the multivariate calibration model (PLS model) can be complex, especially regarding spectra pretreatment. It is true, however, that once solid calibrants have been established, they can be kept for a long time and reused. In addition, infrared spectroscopy is an inexpensive technique, whereas XRF spectroscopes are not common equipment in laboratories.

Generally speaking, the objectives set at the beginning of this work can be said to have been largely met. On the way, however, several new doubts and questions have come up which may serve as the starting point for another future work. Amongst the challenges facing the future the following should be highlighted:

- The greater application of chemometrics in data treatment, for the simultaneous analysis of variables measured in different compartments. In this manner, it would be possible, for example, to relate what is happening in sediment to what is happening in water and to reach common conclusions more effectively and reliably.

- Collection and analysis of suspended particles, which would enable a mass balance of metal pollutants to be performed and, in turn, facilitate analysis of biogeochemical cycles in the river estuary.
- Optimisation of a preconcentration method, as in some cases of water analysis we were close to or below the detection limit. Alternatively, the use of passive sampling, DGT (*Diffusive Gradients in Thin films*), for example.

In the year 2000 the broadest ranging and most specific programme of measures relating to water ever known in Europe, the *European Water Framework Directive, EWFD,* was implemented across the Community. In compliance with the Directive, Member States of the European Union must implement the Directive and ensure the "Good Ecological Status, (GES)" of their waters by the year 2015.

Amongst the water bodies mentioned in the Directive, river estuaries are of special importance, not only because of their notable social and economic value but also because of their significant ecological value. The Nerbio-Ibaizabal river estuary suffered the negative effects of the surrounding area's economic development for many years, in which time chemical pollution accumulated endlessly in the estuary water and sediment. As a result of a general plan to restore the river estuary, however, the situation is gradually improving.

As part of the implementation of the WFD, surveillance monitoring was carried out in the Nerbio-Ibaizabal river estuary, with water and sediment samples collected every 3 months between April 2008 and October 2010. Trace element concentrations in the sediment were determined, along with several other physicochemical properties.

The datasets obtained in these three years, as well as similar data gathered by our research group between January 2005 and January 2008, were used in their entirety. The whole data matrix was analysed using different methods (statistical, graphic and chemometric techniques, amongst others) with a view to assessing the spatial and temporal variability of pollution and identifying possible pollution sources, amongst various other objectives.

At the same time, with respect for green chemistry, cleaner, safer and faster alternative analytical methods based on non-destructive spectroscopic techniques were developed for the determination of trace elements in sediment.