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Review

Comparison of *in situ* sediment remediation amendments: Risk perspectives from species sensitivity distribution[☆]L. Albarano^{a, b}, G. Lofrano^c, M. Costantini^b, V. Zupo^b, F. Carraturo^a, M. Guida^{a, c}, G. Libralato^{a, *}^a Department of Biology, University of Naples Federico II, Complesso Universitario di Monte Sant'Angelo, Via Cinthia 21, 80126, Naples, Italy^b Department of Marine Biotechnology, Stazione Zoologica Anton Dohrn, Villa Comunale, 80121, Napoli, Italy^c Centro Servizi Metrologici e Tecnologici Avanzati (CeSMA), Complesso Universitario di Monte Sant'Angelo, Via Cinthia 21, 80126, Naples, Italy

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ABSTRACT

Contaminated sediment is a major issue for aquatic environments, but attention must be kept even during remediation activities that can negatively affect resident biota especially when applied *in situ*. For the first time, the species sensitivity distribution (SSD) approach was applied to amendments used for *in situ* sediment remediation considering 39 papers including both freshwater (F) and saltwater (S) effect data (i.e. n = 17 only F, n = 19 only S, and n = 3 both F and S). Toxicity data related to the application of activated carbon (AC), nano-Zero-Valent-Iron (nZVI), apatite (A), organoclay (OC) and zeolite (Z) were collected and analyzed. SSD curves were constructed by lognormal model providing comprehensive comparisons of the sensitivities of different species to the relative testing methods. Results indicated that Bacteria were the most sensitive group of testing organisms, while Crustaceans were the less sensitive. The hazardous concentration for 5% of the affected species (HC5) were derived to determine the concentration protecting 95% of the species. OC, A and Z presented both acute and chronic toxicity. The HC5 values in descending order are: AC (4.79 g/L) > nZVI (0.02 g/L) > OC, A and Z (1.77E-04 g/L). AC and nZVI can be considered safer than OC, A and Z in sediment remediation activities, even if *in situ* long-term effects remained still underexplored.

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1. Introduction

Sediment is a fundamental and integrated part of water bodies. It is composed of soluble and insoluble matter, which can be naturally transported from land to ocean, due to inland soil and coastal erosion and windblown dust (Brils, 2008). Pollution is the greater ecological issue due to various discharged toxic substances that can be accumulated in sediment acting as a source of contamination (Arizzi Novelli et al., 2006; Lofrano et al., 2016; Pougnat et al., 2014). Contaminated sediment can strongly impact on aquatic ecosystems, especially in presence of harbors and marinas, embayment, and off coastal areas where commercial and industrial port activities, human settlements and tourism are increasingly widespread (Nikolaou et al., 2009; Lofrano et al., 2016). During dredging activities or natural resuspension phenomena (i.e.

adverse weather conditions), accumulated contaminants could be released from sediment to the water column influencing the survival and fitness of aquatic biota and potentially human health (Arizzi Novelli et al., 2006; Mamindy-Pajany et al., 2010). Thus, the first step to deal with this issue must be the reduction/removal of toxic compounds considering both *in situ* and *ex situ* treatments using different chemical, thermal and biological methods (Gomes et al., 2013; Lofrano et al., 2016; Mueller et al., 1996). Nevertheless, these techniques for the remediation of polluted environments could negatively affect the resident biota, especially when applied *in situ* (Albarano et al., 2020; Libralato et al., 2018; Lofrano et al., 2018). Information about their (eco-)toxicity role impacting on aquatic environment are scarce, making difficult to choose the best potential technology for *in situ* remediation (Lofrano et al., 2016). The consequences of treatment activities on aquatic environment are generally considered as secondary effects (Libralato et al., 2008; Rakowska et al., 2012), while current literature still does not describe any potential undesired long-term effect, and to the best of our knowledge an overview about the different sensitivity of

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aquatic species after their administration including laboratory and field scale applications is not currently available.

This review investigated the potential effects related to the use of amendments in sediment remediation considering the sensitivity of model species from both freshwater and saltwater environments. The species sensitivity distribution (SSD) approach was used to better understand the taxonomic differences in species sensitivity for each remediation method. For the first time, the SSD analysis was evaluated from an updated toxicity database and shown as a cumulative probability distribution for multiple species. SSD curve describes the variation in sensitivity among a set of species toward a contaminant or mixture of contaminants by a statistical or empirical distribution function (Posthuma et al., 2002). The use of this method was proposed for the first time by Kooijman (1987) and later enhanced by further studies (Aldenberg and Jaworska, 2000; Posthuma et al., 2002; Newman et al., 2000; Aldenberg and Slob, 1993; Wagner and Løkke, 1991). Generally, SSDs are generated from laboratory-derived toxicity data offering protection for a wider range of organisms in the field (Hose and Van Den Brink, 2004). The aim of SSDs is to calculate the toxicant concentration affecting a specific number of species usually identified as the hazardous concentration (HC) impairing the 5% (HC5) of organisms, thus the protective concentration (PC) for the 95% of species (PC95) can be calculated as well (Posthuma et al., 2002; Newman et al., 2000). Amendments were selected from previous review papers (Libralato et al., 2018; Lofrano et al., 2018) and were namely activated carbon (AC), nano Zero Valent Iron (nZVI), organoclay (OC), apatite (A) and zeolite (Z).

2. Toxicity data identification, collection and management

Toxicity data about AC, nZVI, OC, A and Z were collected from various sources: Google scholar, National Center for Biotechnology Information (NCBI), and Scopus (last update July 30, 2020). We identified and reviewed 56 papers, but only 39 were selected concerning freshwater and saltwater sediment up to the end of July 2020. The other 17 were eliminated for two main reasons: either i) the tested species were of non-aquatic origin; or ii) the amendments didn't show toxicity on aquatic species concerned (see Table S1). Among the investigated papers 17 (43%) focused on freshwater species, 3 (9%) both freshwater and saltwater environment and approximately 48% only seawater species. Literature was reviewed in order to extract several information as summarized in Table 1 including: taxonomy, endpoints (i.e. mortality (M), reproduction (R), growth inhibition (IG), biota-sediment accumulation factor (BSAF) and bioaccumulation reduction (BR)), exposure time, concentrations, effects of amendments (direct contact) or elutriates (%), and water quality parameters. Units of measures for all amendments (i.e. AC -% sediment dry weight; nZVI -mg/L; OC, A, and Z - g/L) were changed in g/L. For AC, a density of 480 g/L activated carbon was considered (ASTM D2854-89). Since toxicity data related to A, OC and Z amendments were scarce (OC, n = 2; Z, n = 9; A, n = 1), they have been considered as one group (ASTM, 1989).

According to Table 1, we detected nine concentrations (4.8, 7.2, 8.2, 9.6, 12.0, 16.3, 19.2, 24.0 and 36.0 g/L) for AC, six (0.0002, 0.0004, 0.0013, 0.008, 0.050 and 0.5 g/L) for nZVI and eight (0.0001, 0.004, 0.005, 0.1, 0.5, 1, 2 and 350 g/L) for OC, A and Z. Due to the limited number of data, both freshwater and saltwater effect data were considered as a whole targeting the potential risk to aquatic organisms. All the difference existing in the considered materials (such as diameter and morphology) were not explicitly discussed being already included in the reviewed database. Focusing on toxic effects of AC, five taxonomic groups have been tested, for a total of

19 species. Specifically, Bacteria, Annelids, Crustaceans, Molluscs and Fishes were represented with 2, 4, 5, 5 and 3 species, respectively. About nZVI, tested species were 15 including five taxonomic groups (Bacteria, n = 6; Algae, n = 4), Molluscs (n = 3), Crustaceans (n = 1) and Fishes (n = 1)). About OC, A and Z, we identified twelve testing species belonging to three taxonomic groups (Bacteria (n = 1), Crustaceans (n = 5) and Fishes (n = 6)). Toxicity data for AC, nZVI, OC, A and Z were comparable in size and more than 50% of organisms were from Molluscs and Crustaceans for AC, Bacteria and Algae for nZVI, Crustaceans and Fishes for OC, A and Z.

Besides the values to generate SSD curves, data about temperature (T), pH, salinity (S), total organic carbon (TOC), dissolved organic carbon (DOC), chemical oxygen demand (COD), total ammonia nitrogen (TAN) and hardness (H) were collected. Their amount were highly insufficient to go further with data analysis like quantitative structure activity relationship or character-activity relationships (Mu et al., 2014; He et al., 2017; Zhao et al., 2018). AC tests were mainly carried out at room temperature (62.5%) with pH value ranging from 5.6 to 8.6. nZVI experiments were performed between 16 °C–25 °C and pH 5–9. OC, A and Z toxicity were analyzed at increasing temperatures (15 °C–37 °C) and with pH of 7–8.

2.1. Data organization

When more than one toxicity data was registered for the same species (*N. arenaceodentata*, *I. galbana*, *D. magna*, *E. coli* and *D. rerio*), the geometric mean was calculated and used as the estimate for this species as suggested by (Kooijman, 1987). Compiled data were elaborated considering two approaches (Fig. 1): i) raw data (RD) (method 1); and ii) predicted data (PD) (method 2).

About method 1, toxicity data have been shown as raw data without any further processing as they were collected from the 39 reviewed papers. About method 2, average concentrations have been calculated for each amendment: 1) 14.9 g/L for AC; 2) 0.0933 g/L for nZVI, and 3) 44.2 g/L for OC, A and Z. These PD values have been determined for each specific concentration using a proportional calculation (for more details see Supplementary Materials, Table S3). The RD and PD were used as the effect metrics adapted to SSD according to (Van Vlaardingen and Verbruggen, 2007) and the species sensitivity was measured accordingly.

2.2. Data treatment and statistical analysis

The toxicity values were log-transformed according to (Burmaster and Hull, 1997; Leo Posthuma, Glenn W. Suter II, 2002; Newman et al., 2000) using Equation (1):

$$\chi = \log_{10}(RD \text{ or } PD) \quad (1)$$

The associated risk was visualized as cumulative distribution function as defined in Equation 2

$$y = \sum_{i=1}^K n_i \quad (2)$$

where y is the cumulative probability of species and n_i is the absolute frequency of single RD or PD value.

The distribution model was fitted to toxicity data points and evaluated using the χ^2 goodness of fit with the adjusted coefficient of determination R^2 (Adj- R^2). The median hazard concentration (HC50) and the HC affecting the 5% of species were calculated according to (Aldenberg and Slob 1993), using Equation (3):

Table 1

Sediment treatment, group of organisms, species, time of exposure, concentrations, endpoints, effects and references of negative impact of contaminated sediment restoring. Abbreviations: M = Mortality, BSAF = biota-sediment accumulation factor, BR = Bioaccumulation reduction, IG = inhibition growth and R = reproduction, n.e. = not effect, T = Temperature, S = Salinity, DOC = dissolved organic carbon, COD = chemical oxygen demand, TOC = total organic carbon, TAN = total ammonia nitrogen, H = hardness, n.a. = not available.

Sediment treatment	Group of organisms	Species	Exposure Time (days; *h; +min)	Concentrations (g/L)	Endpoints	Effects (%) of amendments/elutriates	Water quality parameters (T = °C, S = ppt, TOC-DOC-COD-TAN-H = mg/L)	References
AC	Bacteria	<i>Escherichia coli</i>	0.5*	4.8	M	79.5	pH = 6.8	Van Der Mei et al. (2008)
		<i>Raoultella terrigena</i>	0.5*	4.8	M	65.5	pH = 6.9	Van Der Mei et al. (2008)
	Annelids	<i>Nereis diversicolor</i>	28	9.6	BSAF	2	T = 22	Cornelissen et al. (2006)
		<i>Limnodrilus</i> spp	28	7.2	BR	94	T = 20	Jonker et al. (2004)
		<i>Neanthes arenaceodentata</i>	28	16.3	BR and IG	50 and 73	T = 20	Millward et al. (2005)
		<i>Arenicola marina</i>	10	36	M	70	T = 20, pH = 6.15–8.61	Lillicrap et al. (2015)
		<i>Macoma balthica</i>	28	16.3	BR	76	T = 13	McLeod et al. (2007)
	Molluscs	<i>Lymnaea stagnalis</i>	41	8.2	BR	37.3	pH = 5.6–6.5, RT, DOC = 35–40	Lewis et al. (2016)
		<i>Corbicula fluminea</i>	28	12	BR	95	T = 13	McLeod et al. (2008)
		<i>Potamopyrgus antipodorum</i>	28	9.6	R	9.7	T = 16, pH = 8.0, DOC = 9.4, COD = 26.5, TAN = 5.35	Stalter et al. (2010)
		<i>Meretrix meretrix</i>	28	24	IG	36.4	T = 20, pH = 8.05	Zheng et al. (2018)
	Crustaceans	<i>Daphnia Magna</i>	4	19.2	BR	4	T = 20	Jonker et al. (2009)
		<i>Asellus aquaticus</i>	28	19.2	IG	36	T = 20	Kupryianchyk et al., 2011
		<i>Corophium volutator</i>	28	19.2	IG	50	T = 20	Jonker et al. (2009)
		<i>Leptocheirus plumulosus</i>	10*	16.3	BR	70	T = 20	Millward et al. (2005)
		<i>Tisbe battagliai</i>	72*	36	M	60	T = 20, pH = 6.15–8.61	Lillicrap et al. (2015)
	Fishes	<i>Pimephales promelas</i>	10	24	M	43.8	T = 25, pH = 8.2	He et al. (2012)
		<i>Dicentrarchus labrax</i>	28	4.8	M	76.4	T = 21.8, pH = 8.0 S = 37, TAN = 0.18	Aly et al. (2016)
		<i>Gambusia affinis</i>	10	36	IG	62.2	T = 20	Casini et al. (2006)
nZVI	Bacteria	<i>Escherichia coli</i>	0.5* 1*	0.008	M	70 80	T = 32, pH = 7.2 T = 30, pH = 5–7.4	Lee et al. (2008)
		<i>Bacillus subtilis</i> var. <i>niger</i>	5+	0.0002	M	20	T = 20, pH = 9	Auffan et al. (2008)
		<i>Pseudomonas fluorescens</i>	5+	0.0002	M	100		Diao and Yao (2009)
		<i>Vibrio fischeri</i>	2	0.0002	IG	87.2	T = 20, pH = 5–9, DOC = 0.01	Qiu et al. (2013)
		<i>Dehalococcoides</i>	2	0.5	IG	98.2	T = 22, pH = 8.1	Xiu et al. (2010)
		<i>Microcystis aeruginosa</i>	30	0.5	IG	92	T = 30, TOC = 18.75, TAN = 1.73	Su et al. (2018)
	Algae	<i>Isochrysis galbana</i>	4	0.0002	IG	50 and 60	pH = 7.5, RT	Keller et al. (2012)
		<i>Dunaliella tertiolecta</i>	4	0.0013	IG	53		Othman (2018)
		<i>Thalassiosira pseudonana</i>	4	0.0004	IG	51		
		<i>Pseudokirchneriella subcapitata</i>	4	0.008	IG	47		
	Molluscs	<i>Lymnaea stagnalis</i>	41	0.0002	R	59.4	pH = 5.6–6.5, DOC = 35–40	Lewis et al. (2016)
		<i>Mytilus galloprovincialis</i>	28	0.05	IG	14	T = 18, pH = 8.0, S = 30	Coppola et al. (2019)
		<i>Mytilus galloprovincialis</i>	2	0.008	IG	60	T = 16, pH = 6–7, DOC = 0.08	Kadar et al. (2010)
		<i>Mytilus galloprovincialis</i>	28	0.0004	M, IG and R	60, 58 and n.e.	RT	Keller et al. (2012)
	Crustaceans	<i>Daphnia Magna</i>	28	0.0004	R	60, 58 and n.e.	RT	Jaafar et al. (2018)
	Fishes	<i>Oryzias latipes</i>	10	0.0002 0.05	IG M	30 90	T = 25, pH = 7–7.6, H = 200 T = 26, pH = 7	Li et al. (2009)
								Chen et al. (2011)
A, Z and OC	Bacteria	<i>Escherichia coli</i>	0.5*	0.004	M and IG	52.9 and 60	T = 37, pH = 7.2	Rieger et al. (2016)
	Crustaceans	<i>Hyalella azteca</i>		0.1	M	67.5	T = 25	Paller and Knox (2010)
		<i>Leptocheirus plumulosus</i>		0.1	M	21.3		
		<i>Americamysis bahia</i>	2	0.5	M	47	T = 15	Burgess et al. (2004)
		<i>Ampelisca abdita</i>	2	0.5	M	48		

(continued on next page)

Table 1 (continued)

Sediment treatment	Group of organisms	Species	Exposure Time (days; *h; +min)	Concentrations (g/L)	Endpoints	Effects (%) of amendments/elutriates	Water quality parameters (T = °C, S = ppt, TOC-DOC-COD-TAN-H = mg/L)	References
		<i>Paranephrops planifrons</i>	10	350	IG	33.3	T = 15, pH = 7, TAN = 2	Parkyn et al. (2011)
	Fishes	<i>Danio rerio</i>	3	0.0001	IG and M	96.5 and 66	pH = 7, RT	Palcic et al. (2020)
		<i>Oncorhynchus mykiss</i>	0.5	1	BR	86.6	T = 14, pH = 8	Aly et al. (2016)
		<i>Dicentrarchus labrax</i>	28	0.1	M	83.3	T = 14, pH = 7.4	Ukar et al. (2017)
		<i>Gambusia affinis</i>	28	0.1	IG	42.2	T = 21.8, pH = 8.0 S = 37, TAN = 0.18	Aly et al. (2016)
		<i>Oreochromis mossambicus</i>	10	0.005	M	42.8	T = 20	Casini et al. (2006)
		<i>Oryzias latipes</i>	28	2	BR	41.7	T = 29, pH = 7.8, H = 58	James et al. (2000)
			10	0.005	IG	30	T = 26, pH = 7	Chen et al. (2011)

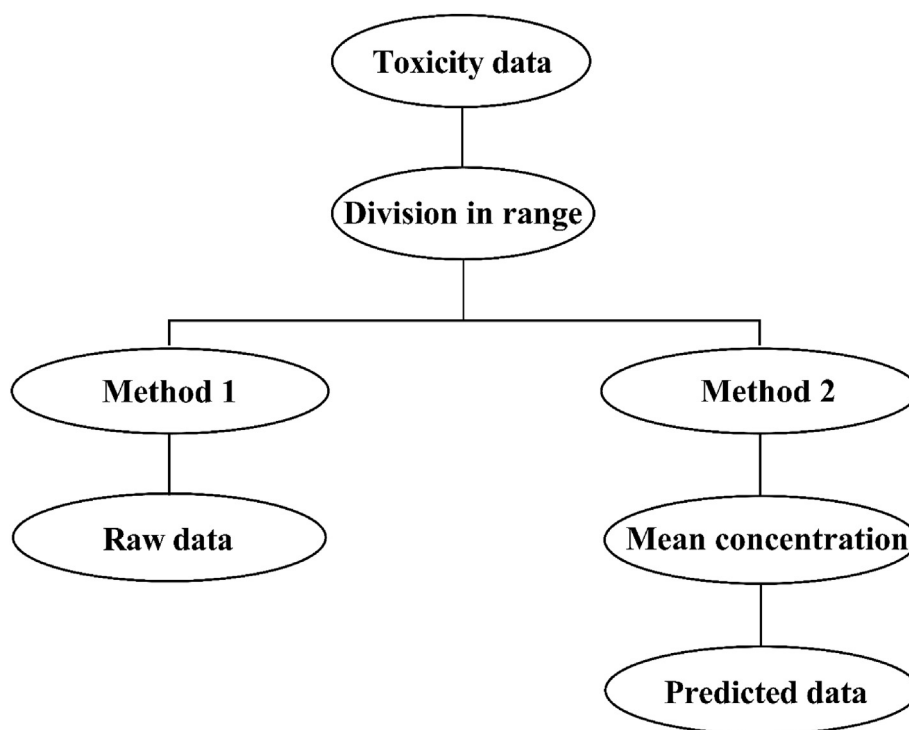


Fig. 1. Flowchart of data-processing.

$$\text{Log}(HC_p) = \mu - \mathcal{K}_p * \sigma \quad (3)$$

where HC_p is hazardous concentration for percentage of the species population, $\mathcal{K}_p$ is Aldenberg extrapolation factor that directly depends of the number of the studied species, μ and σ are the mean and the standard deviation of distribution, respectively.

Data were analyzed by Shapiro-Wilk's (S–W) test for normality and F-test for homoscedasticity (p-value <0.05). For each amendment, statistical significance between different groups of organisms was performed by one-way analysis of variance (ANOVA) followed by Tukey's test for multiple comparisons (p < 0.05). Two fixed factors (groups of organisms vs remediation methods) were crossed by a two-way ANOVA. All statistical analyses were performed using GraphPad Prism Software (version 8.02 for Windows, GraphPad Software, La Jolla, California, USA, www.graphpad.com).

3. Results and discussion

As reported in Table 2, RD and PD are normally distributed for

Table 2

Goodness-fit, Shapiro-Wilk's (S–W) test for normality and F-test for homoscedasticity of total species for three remediation methods. p is the significance level. WR = whole range, MC = mean concentration.

Sediment treatment	Concentrations (g/L)	Adj-R2	S–W	F	p
AC	4.8–36.0 (WR)	0.97	>0.9999	0.11	<0.05
	14.9 (MC)	0.97	0.12	0.06	<0.05
nZVI	0.0002–0.5 (WR)	0.93	0.34	0.98	<0.05
	93.3 (MC)	0.96	0.18	0.49	<0.05
OC, A and Z	0.0001–350 (WR)	0.97	0.14	0.26	<0.05
	44.2	0.97	0.08	0.13	<0.05

AC (p values = >0.9999 and 0.1218, respectively), nZVI (p values = 0.34 and 0.18, respectively), OC, A, and Z (p values = 0.14 and 0.08, respectively). The value of RD and PD show variance homogeneity (homoscedasticity) for AC (p values = 0.11 and 0.06, respectively), for nZVI (p values = 0.98 and 0.49, respectively) and

for OC, A and Z (p values = 0.26 and 0.13). The results indicated that the lognormal distribution fits with most of the groups data points, with Adj- R^2 ranging from 0.79 to 0.98 (p -value < 0.01) (as shown in Table 2 and Table S4).

3.1. The SSDs of AC

Taking into consideration both the entire selection (4.8–36.0 g/L, Fig. 2A) and its mean concentration (14.9 g/L, Fig. 2D), Bacteria were largely more susceptible than other organisms. Specifically, at the 14.9 g/L concentration (Fig. 2D), Bacteria group has shown a significant increase of sensitivity to AC respect to Annelids ($p < 0.05$), Molluscs ($p < 0.05$) and Crustaceans ($p < 0.01$) (see also Table S5). Crustaceans, in particular *D. magna*, exhibited the lowest susceptibility.

Considering the range 4.8–9.6 g/L (Fig. S1A), Bacteria, especially with *E. coli*, the Annelid *Limnodrilus* spp. and the Mollusc *C. fluminea* were the most sensitive species. Furthermore, regarding the respective mean concentration (7.2 g/L, Fig. S1D) Bacteria, with *E. coli* and *R. terrigena*, showed the highest significant sensitivity to AC respect to Molluscs ($p < 0.05$) (Table S5).

Given the range 12.0–16.3 g/L (Figura S1B), Annelids group, as reported in Table S2, has displayed a significant sensitivity respect to Molluscs ($p < 0.05$), that in turn were statistically significant respect to Crustaceans ($p < 0.01$). However, when the 13.4 g/L mean concentration has been viewed, Molluscs group were the highest susceptible respect to other groups of species, but not statistically significant (Fig. S1E).

Analyzing both the range 19.2–36.0 g/L (Fig. S1C) and its respective mean concentrations (26.4 g/L, Fig. S1E) the Fish *G. affinis*

has shown a high sensitivity despite the p -values were greater than 0.05.

As displayed in Fig. 2A, some species within the same taxonomic group responded differently showing a variable sensitivity. The toxicity data of AC showed that the polychaetae *N. diversicolor* and *Limnodrilus* spp. were the least and most sensitive species among the Annelids, respectively. In particular, cumulative probability of *Limnodrilus* spp. exceeded 90% in SSD curves, whereas that of *N. diversicolor* resulted to be of 2%. Among Molluscs group, *M. meretrix* and *L. stagnalis* were the least sensitive species (with a cumulative probability of approximately 37%), whereas *C. fluminea* with a cumulative probability of 95% resulted the most sensitive species. Furthermore, *D. magna* and *T. battagliai* were respectively the least and most sensitive species among the Crustaceans group. Specifically, cumulative probability of *T. battagliai* was 60% in SSD curves, whereas that of *D. magna* resulted to be of 4%. Probably, considering that the diameter of the feeding chaetoceros was generally 7–9 mm, this substantial variability was due to possible differences in digestive biology of species (Cornelissen et al., 2006; Jonker et al., 2004, 2009; Millward et al., 2005; Zheng et al., 2018).

3.2. The SSDs of nZVI

Considering the raw data, also for the nZVI methods the Bacteria, specifically *P. fluorescens*, were largely more susceptible than others organisms (Fig. S2). Given the whole range (0.0002–0.5 g/L, Fig. 2B), Bacteria, specifically *P. fluorescens* and *V. fischeri*, were statistically significant compared to Algae ($p < 0.0001$), Cusaceans ($p < 0.001$), Molluscs ($p < 0.001$) and Fishes ($p < 0.001$) (Table S6). When taking into consideration the predicted data (Figs. S2C–D;

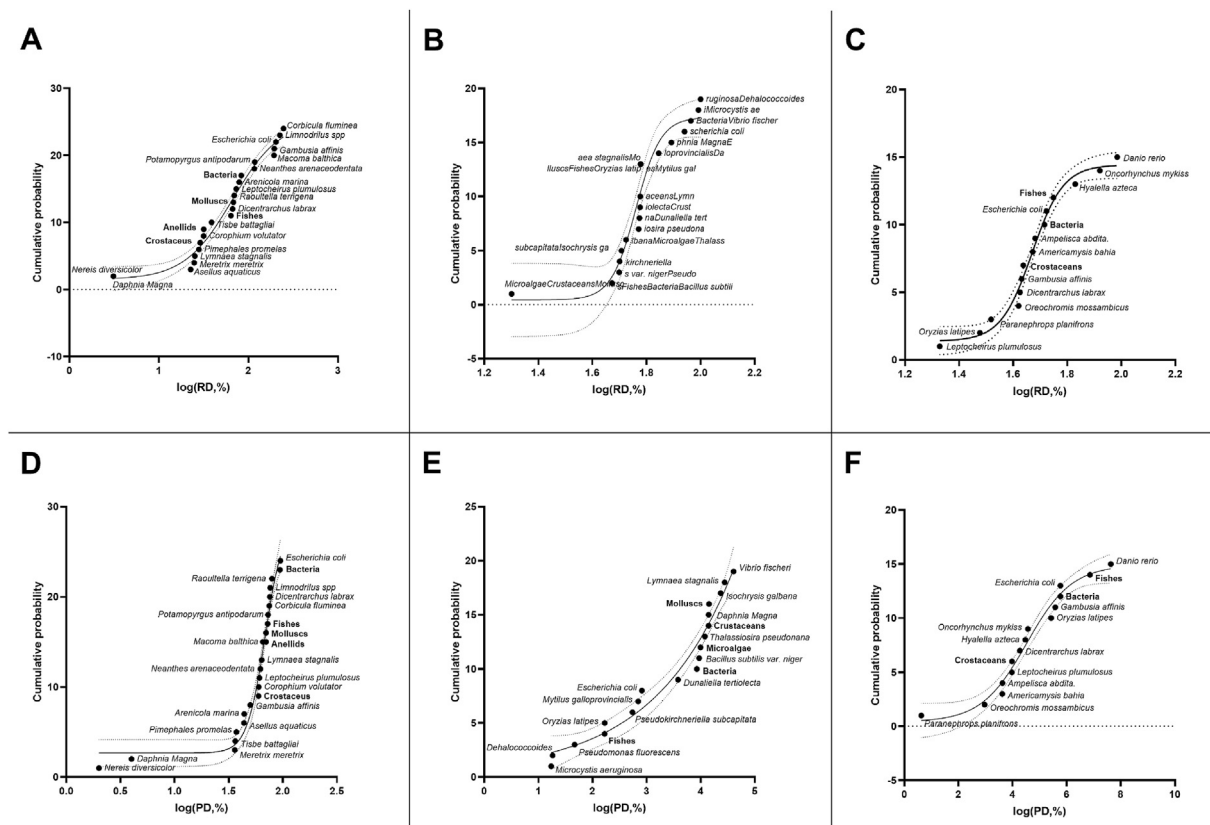


Fig. 2. Species sensitivity distribution of different groups species to AC, nZVI, OC, A and Z. The data in (A–C) are represented as raw data (RD) collected respectively from 4.8 to 36.0 g/L (AC), 0.0002–0.5 g/L (nZVI) and 0.0001–350 g/L (OC, A and Z); the data in (D–F) are reported as predicted data (PD) calculated respectively 14.9 (AC), 0.0933 (nZVI) and 44.2 g/L (OC, A and Z).

Fig. 2E), Bacteria, with *E. coli*, *P. fluorescens* and *V. fischeri*, and the Molluscs, in particular *L. stagnalis*, were among the most affected species despite the p-values were >0.05 ; whereas the Fishes and Algae were the less impacted class of organisms. Furthermore, Bacteria group showed a variable sensitivity. *Bacillus subtilis* var. *niger* was the least sensitive, whereas *P. fluorescens* and *V. fischeri* were the most sensitive species among Bacteria. Their cumulative probability in SSD curves were 20%, 100% and 87.2%, respectively. *P. fluorescens* and *V. fischeri* are gram-negative bacteria, which are more sensitive to environmental stress respect to *B. subtilis*, which is a gram-positive bacterium (Diao and Yao, 2009). Moreover, probably *A. fischeri* and *P. fluorescens* showed similar effects because they were equally sensitive to metal ions (Abbas et al., 2018; Abbondanzi et al., 2003).

Analysing the range 0.008–0.5 g/L (Fig. S2B), Bacteria group showed the highest significant sensitivity to nZVI when compared to Algae ($p < 0.001$) and Molluscs ($p < 0.05$) (see Table S6). Algae species were statistically significant respect to Fishes ($p < 0.001$), that in turn displayed high susceptibility respect to Molluscs ($p < 0.01$, Table S6).

3.3. The SSDs of OC, A and Z

Finally, for the OC, A and Z methods the Fishes, in particular *D. rerio* and *O. mykiss* species, were resulted the most susceptible considering the raw data (Figs. S3A–B), but not statistically significant (Table S7). When also given the predicted data (Figs. S3C–D), the Fish species, especially *D. rerio*, displayed a highest sensitivity to OC, A and Z remediation methods. Moreover, as shown in Table S7, when analyzing 44.2 g/L mean concentration (Fig. 2F) Fishes group were statistically significant respect to Crustaceans ($p < 0.05$) and Bacteria ($p < 0.01$), that in turn displayed high susceptibility respect to Crustaceans ($p < 0.0001$) (see also Table S7). Moreover, Fishes group showed a variable sensitivity. *O. latipes* was the least sensitive species (with a cumulative probability of about ~30%), but *D. rerio* displayed the highest sensitivity with a cumulative probability of 96.5%. Probably, the triazoles leaching from the zeolite channels can cause the Z toxicity. De La Paz et al. (2017) demonstrated that triazoles can inhibit hatching through affecting the hatching enzyme or impairing the release of ZHE1 enzyme.

Furthermore, taking into consideration both raw and predicted data, Crustaceans, in particular *L. plumulosus* and *P. planifrons*, have proven to be the less sensitive species (Fig. 2C; F).

3.4. Whole comparison of SSDs

As shown in Fig. 3, SSDs of different remediation methods based on the total species were constructed and the relationship of sensitivity between individual group species and total species was investigated for all amendments considering both raw and predicted data (Fig. 3).

Considering the raw data (Fig. 3A), the curves of remediation methods were almost overlapping with exception of Bacteria that showed the significant increase of sensitivity to nZVI respect to OC, A and Z ($p < 0.05$) and Crustaceans group that were statistically significant to AC respect to nZVI ($p < 0.01$) (see Table S8).

At intermediate concentrations and considering predicted data, the AC curve method shifted to the left (Fig. 3B). The adverse effect of nZVI was intermediary between all amendments, whereas the OC, A and Z curve shifted on the right showing higher toxicity for all studied species. Only Fishes group displayed a significant increase of sensitivity to OC, A and Z respect to AC ($p < 0.05$) and nZVI ($p < 0.05$) (Table S8).

The AC is more toxic to Fishes than the other four taxonomic groups of analyzed species (with the HC5 calculated at 3.44 g/L, CI = 0.18–8.97; Table 3), followed by Annelids, Molluscs and Crustaceans. The HC5 value of Crustaceans was found to be more than 3 times higher (11.16 g/L, CI = 5.92–14.81, Table 3) than that measured in Fishes. Similarly, it occurred for HC50. The decreasing sensitivity is: Fishes > Annelids > Molluscs > Crustaceans. Only for Bacteria, the HC5 and HC50 values were not calculated because just one concentration was available. nZVI method has a highest impact on Algae (8.58E-02 g/L, CI = 2.49E-03 - 0.35, Table 3) respect to Molluscs and Bacteria. The HC5 value of Molluscs was found to be more than 2 times higher than that of Algae. For Crustaceans and Fishes, HC5 and HC50 were not calculated because just one concentration was available. The decreasing sensitivity is: Algae > Bacteria > Molluscs.

About OC, A and Z methods, Fishes were the most affected species with HC5 of 9.46E-05 (CI = 3.78E-05 - 1.4E-03, Table 3). HC5 of Crustaceans group results to be more than 59 times higher than

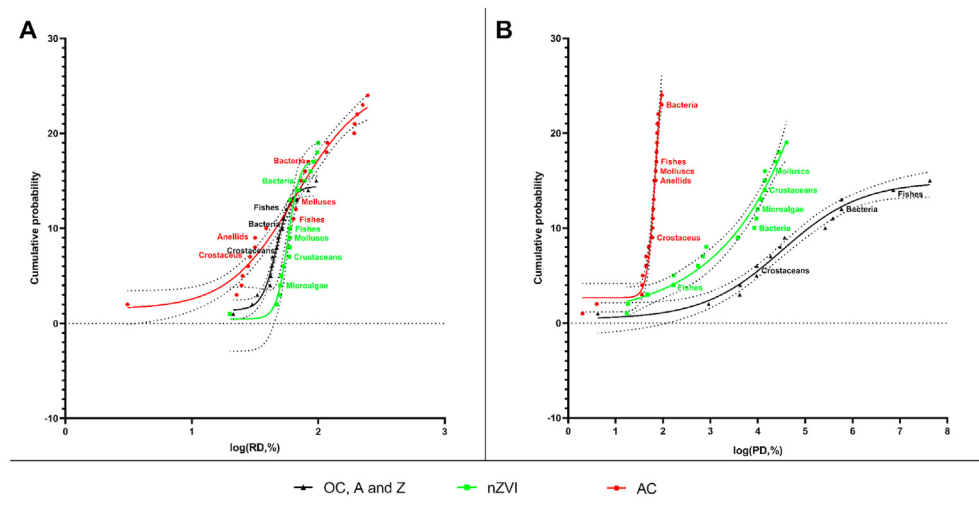


Fig. 3. SSD curves for total species exposed to different remediation methods. The data in (A) are represented as raw data (RD) and the findings in (B) are reported as predicted data (PD) for AC (red line), for nZVI (green line) and for OC, A and Z (black line). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 3

The calculated hazard concentration at 5% (HC5) and 50% (HC50) of species (including their CI (Confidence interval)) of Bacteria, Annelids, Molluscs, Crustaceans, Algae, Fishes and total species for three remediation methods. n.a. = not available.

		HC5 (g/L)	CI	HC50 (g/L)	CI
AC	Bacteria	n.a.	n.a.	n.a.	n.a.
	Annelids	4.88	1.09–8.82	14.59	8.11–26.23
	Molluscs	6.56	2.76–9.64	14.19	9.87–20.39
	Crustaceans	11.16	5.92–14.81	19.66	15.06–25.65
	Fishes	3.44	0.18–8.97	17.30	6.12–48.87
	Total	4.74	2.96–6.53	14.00	10.89–17.98
nZVI	Bacteria	1.3E-02	2.43E-05 - 0.26	8.29	0.53–130.1
	Algae	8.58E-02	2.49E-03 - 0.35	1.15	0.29–4.65
	Molluscs	4.15E-02	5.46E-07 - 0.53	1.87	0.07–51.44
	Crustaceans	n.a.	n.a.	n.a.	n.a.
	Fishes	n.a.	n.a.	n.a.	n.a.
	Total	0.02	2.9E-03 - 0.08	1.92	0.67–5.46
OC, A and Z	Bacteria	n.a.	n.a.	n.a.	n.a.
	Crustaceans	5.59E-03	1.18E-05 - 0.08	1.38	0.10–18.44
	Fishes	9.46E-05	3.78E-05 - 1.4E-03	2.93E-02	2.6E-03 - 0.33
	Total	1.77E-04	5.69E-06 - 1.6E-03	1.21E-01	0.02–0.71

that of Fishes (5.59E-03, CI = 1.18E-05 - 0.08, see Table 3). Moreover, also in this case, for Bacteria, the HC5 and HC50 values were not calculated because just one concentration was available. As reported in Table 3, when considered the impact of both AC, nZVI, OC, A and Z methods on total species, OC, A and Z was the most toxic with the HC5 value of 1.77E-04 (CI = 5.69E-06 - 1.6E-03), followed by nZVI and AC. Specifically, the HC5 value of AC is much greater than that measured for OC, A and Z. In this case, the decreasing risk is: OC, A and Z > nZVI > AC (Table 3).

Focusing on HC5 and HC50 of remediation methods for each taxonomic group of species and total species, the toxicity profiles have been established on the basis of OECD criteria (2006) OECD, 2006. According to the United Nations Globally Harmonised System for Classification and Labelling (UNECE, 2003), AC and nZVI methods have been identified as "no-hazardous" to aquatic environment. However, OC, A and Z remediation displayed higher toxicity levels (i.e. acute and chronic) for species belonging to both saltwater and freshwater environments representing a potential risk to the aquatic life (i.e. class 3). Scarce information exists on the toxicity mechanisms associated with the use of OC, A and Z. The active biomonitoring studies indicated that the biopolymers used in some capping bound sand grains and other particles in a viscous matrix that appeared to entrap and possibly suffocate burrowing organisms (Paller and Knox, 2010). Janer et al. (2013) demonstrated that six types of nanosized clays, specially organoclay, were also able to induce apoptosis and to spread in cytoplasmic vesicles of the exposed cells at low concentrations. The toxicity impact of Z can be probably due to substances leaching from the zeolite channels, which are able to cause an increase of the specific enzymatic activities (Casini et al., 2006; Aly et al., 2016).

4. Conclusions

The present study investigated the toxicity of different remediation methods towards saltwater and freshwater species according to the species sensitivity distribution approach. When RD values were considered Bacteria group showed the higher sensitivity to nZVI respect to OC, A and Z, and Crustaceans to AC compared to nZVI. Taking into consideration the PD, Fishes group were ranked as more vulnerable to OC, A and Z compared to AC and nZVI. On the basis of HC5 and HC50, AC, OC, A and Z presented the higher adverse effects on Fishes. nZVI was more at risk for Algae, followed by Bacteria and Molluscs. In general, AC and nZVI were ranked as safer (i.e. at low risk) than all other amendments on the basis of GHS criteria. OC, A and Z proved to significantly present

both acute and chronic toxicity. The risk of the considered amendments listed in a descending order are: (OC, A and Z) > nZVI > AC. Further investigations are necessary to understand the long-term effects of AC and nZVI after *in situ* application on the potential exposed biota.

Credit author statement

Luisa Albarano: Conceptualization, Methodology, Software. Giusy Lofrano: Data curation, Writing- Original draft preparation. Maria Costantini: Conceptualization, Investigation. Valerio Zupo: Methodology, Software. Federica Carraturo: Data curation. Marco Guida: Conceptualization, Supervision. Giovanni Libralato: Conceptualization, Supervision, Writing- Reviewing and Editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envpol.2020.115995>.

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