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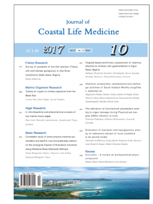

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Toxicity of copper on marine organisms from the Black Sea

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ABSTRACT

Objective: To investigate the effect of copper on *Crangon crangon* (Linnaeus, 1758) (*C. crangon*) and *Syngnathus acus* (Linnaeus, 1758) (*S. acus*) from Black Sea.
Methods: The acute toxicity of copper in water with clean sediment to *C. crangon* and *S. acus* from Sinop Peninsula of the Black Sea was evaluated by static 10-day and 21-day bioassays.
Results: Mortality of both organisms increased with increase in concentrations of copper. The results showed that *S. acus* was more sensitive to copper than *C. crangon*.
Conclusions: In the present study, both *C. crangon* and *S. acus* have been shown to be a suitable test species to assess heavy metal toxicity using static 21-day and 10-day bioassays.

1. Introduction

Heavy metals, widely used in industry, have been accumulated in increasing quantities in recent years as contaminants in all components of the biosphere especially in marine ecosystems[1,2]. Heavy metals have the potential to be lethally dangerous even in small quantities[3]; thus, it is very important to understand the mechanisms of entry of contaminants into marine waters and their availability[4] and potential effects on biota and ecosystems[5].

With respect to the Marine Strategy Framework Directive 2008/56/EC (MSFD), the relevant qualitative descriptor for contaminants for determining Good Environmental Status (GES) is Descriptor 8 which states that ‘concentrations of contaminants are at levels not giving rise to pollution effects’, while Descriptor 8.2.1 describes a specific indicator for pollution effects that ‘Levels of pollution effects on the ecosystem components concerned, having regard to the selected biological processes and taxonomic groups where a cause/effect relationship has been established and needs to be monitored’. Here, research support is expected for a better understanding of the relationship between pressures and their effects and impacts on the marine environment, and for deriving operational indicators for GES assessment. The MSFD is aiming at the protection of the environment against contaminants and is to identify eventual environmental problems which hinder the achievement of GES and the identification of their cause and thus the sources of chemical contamination[6]. This status stresses the necessity to research not only short term toxic effects of these metals but also the long term implications of low levels of exposure on the structure and balance of ecosystems.

The main emphasis of this study is on heavy metal pollution, a subject which is of particular interest because of the essential requirement of organisms for trace quantities of many metals and the fine balance between requirement and excess, disturbance of which results in markedly deleterious effects. Essential metals are components of many biologically important macromolecules and are frequently the active site in these molecules[7]. Cu, for example, is a component in the blood pigment haemocyanin, and in

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cytochrome-c. In addition, free metal ions function as coenzymes in many cellular processes[8,9].

There is still little information on the ways probably by which heavy metals affect the structure of ecosystems as a result of interaction between affected and unaffected species. The present study aimed to investigate the effect on *Crangon crangon* (Linnaeus, 1758) (*C. crangon*) and *Syngnathus acus* (Linnaeus, 1758) (*S. acus*) of copper, which is essential in trace quantities, yet markedly toxic even at quite low concentrations.

2. Materials and methods

2.1. General information concerning *C. crangon*

C. crangon survives for 4 years and reaches a maximum length of 80 mm, maximum weight of 6.464 g. Reproduction period of *C. crangon* is between February and October and all month. Ovigerous females of *C. crangon* are the most abundant in May and September. The size at 50% sexual maturity for females is 53.5 mm in total length off Sinop Peninsula in the Black Sea[10,11]. *C. crangon* has a geographic distribution ranging through the North Sea and Baltic Sea, the Mediterranean Sea, Black Sea and the White Sea. They live largely at depths from 0 to 50 m. The distribution of *C. crangon* is affected by environmental factors such as temperature, habitat characters and varied seasonal migration models[12]. It favours areas of brackish water with mud or sand substrata and strong water movement. Experiments have shown that *C. crangon* is found in its richest abundance in sediment with particles intermediate in size between silt and coarse sand, and this is presumably because this permits the most efficient and rapid burial. *C. crangon* has been identified as a major predator in areas of shallow sea, one of the most abundant members of the macro-fauna and an important component of the food web. There is a change in its diet as the size of the shrimp increases. Small individuals predate meiofauna, particularly ostracods and harpacticoids, also nematodes and benthic foraminifera. Large ones predate macro-fauna, having a significant effect on the population of amphipods, worms, schizopods (*i.e.* Mysidacea and Euphausiacea), snails, young bivalves and young fish. Most of *C. crangon* do not survive in the first year; some individuals, however, survive for 2 or more years, and very occasionally, may attain an age of 5 years and reach a maximum length of about 90 mm.

2.2. General information concerning *S. acus*

S. acus is a fish found in the coastline of Sinop Peninsula on sand and amongst algae and eel-grass. *S. acus* feeds mainly on small crustaceans[13]. The body of greater pipefish is narrow and elongated, of verrucous appearance. The head has a long muzzle over more than half the length of it, and a small bump on the nape of the neck. The greater pipefish has pectoral fins, a dorsal fin and a small caudal fin. Its colour is brown with green or black vertical stripes, but it may vary depending on the environment[14].

2.3. Collection of animals

C. crangon appears during the winter and spring and the number of individuals increases to reach their highest abundance in mid-winter and mid-spring[15]. Specimens of *C. crangon* were collected from Sinop Peninsula in 2013 (Figure 1).

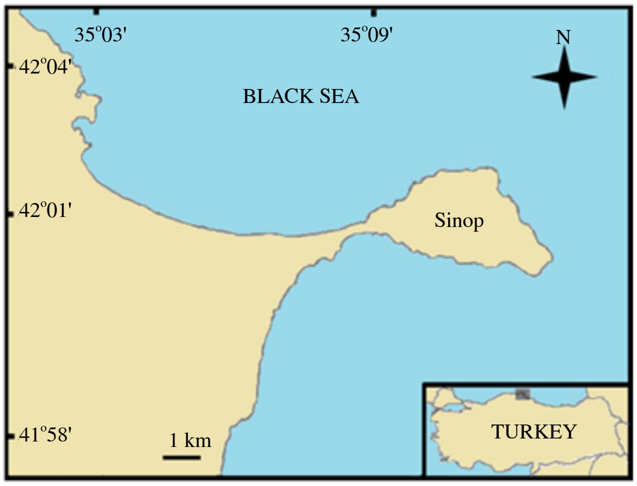

Figure 1. Collection area.

All individuals of *C. crangon* were captured at the depth of 10 m with mud or sand substrata. Specimens were sampled by beam trawls with length of 3 m and 10 mm cod-end mesh size. The specimens clearly recognized as *C. crangon* were separated and total length (TL) (from tip of rostrum to the tip of telson along the mid dorsal line) of each specimen was measured to the nearest 0.1 mm using vernier callipers. They were separately placed in biologically filtered clean seawater with 3 cm depth of the clean sediment into plexiglas experimental stock tanks (30 cm × 30 cm × 20 cm) at temperature of 21 °C. Specimens of *C. crangon* were fed with *Artemia salina*.

Specimens of *S. acus* were collected during spring and autumn in 2013. Specimens were captured by beam trawl with 3 m length and 20 mm cod-end mesh size, at a depth of 10–20 m. The sizes of the brood-stock were 9 to 13 cm. It has been observed that gravid greater pipefish in stock aquarium gave birth a day later (approximately 20–24 h). New-borns seemed to have absorbed the yolk sac. Aquarium experiments have shown that new-borns were benthic, remaining close to the bottom. New-borns were measured with a 0.1 mm precision by callipers and the mean total length was determined as (20.00 ± 0.71) mm (min: 18 mm and max: 22 mm). After they were fed with *Artemia salina* nauplii, which were opened at 30‰ salinity, in the morning and evening for 6 day, the experiment was started with greater pipefish.

2.4. Experimental protocol

Clean sediment was collected from the same area and washed through a 500-μm mesh sieve into a tank to remove any associated macrofauna, and then washed again at least 3 times with clean seawater before used in subsequent experiments[16,17]. Clean sediment was added to the test tanks to create a 3-cm deep layer.

In order to evaluate the effect of copper on these organisms, it is necessary to have understanding of the role of the metal in general and the relationship between species. An excess of the particular metal is likely to have an effect on the balance of biological function of the organisms. Therefore, first of all short term (10 days) experiments were carried out in order to define lethal and sub-lethal concentrations of copper. The results were used as a basis for choosing test concentrations for the further experiments (21-day experiments)[16,17].

Stock solutions of MERC grade chemicals, copper (II) sulphate ($CuSO_4 \cdot 5H_2O$), were prepared in seawater and diluted as required.Three replicated series of concentrations and controls were used and all control and test solutions were aerated[16-23]. Temperature, dissolved oxygen, salinity and pH were measured in all experiments and all replicates, and treatments were applied with the same ecologic factors to reduce any potential physiological stress on the organisms encountered during bioavailability of chemical[16,17]. Test solutions were not changed. Damaged animals were not used[16,17,20].

In all statistical tests, the significance level was set at $P = 0.05$. If data were normally distributed and variances homogeneous, the appropriate parametric test (*e.g.* Students *t*-test) was used, otherwise, non-parametric tests (*e.g.* Mann-Whitney test) were employed. When more than two samples were tested, One-way ANOVA was used. If ANOVA results were significant, the control and treatment means were compared by Tukey test to determine which treatment(s) differed from which[24].

3. Results

The water quality measurements showed that the average temperature of the water was (20 ± 1) ˚C, salinity (17.5 ± 1.0)‰, pH 8.1 ± 0.2 and dissolved oxygen (7.1 ± 0.2) mg/L. These values were not statistically different between the controls and the treatments and replicates. Average size of *S. acus* specimens used in the experiment was (26.80 ± 0.95) mm (minimum and maximum lengths were 24 and 30 mm). The size of the *C. crangon* specimens is showed in Figure 2. There was no mortality in all the controls for all species, indicating that the holding facilities, water, uncontaminated sediment and handling techniques were acceptable for conducting toxicity tests, as required in the standard EPA/COE protocol where mean survival should be ⩾ 90%[25].

Animals were checked daily for mortality. Survival of all species decreased with increasing copper concentrations in seawater. The mean survival values for animals are shown in Figures 3 and 4.

In the present study on the second day, 80% of the shrimp treated at 20 mg/L died. However, at the end of the 13th day, all of the shrimps exposed to 2 mg/L or less of copper were alive. All of the pipefish died at the end of the 3rd day treated at 5 mg/L. The results showed that *S. acus* was more sensitive to copper than *C. crangon*.

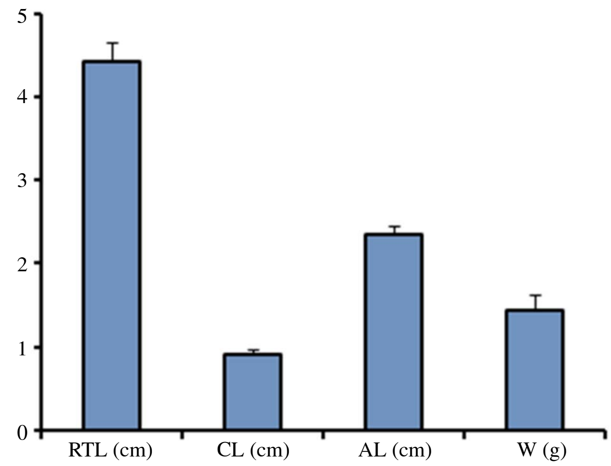

Figure 2. Size of *C. crangon*.
RTL: Total length with rostrum; CL: Carapace length; AL: Length of abdomen; W: weight.

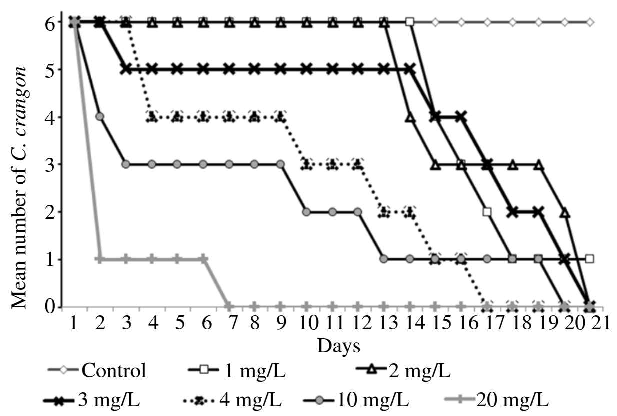

Figure 3. Mean number of *C. crangon* surviving in seawater containing different copper concentrations.

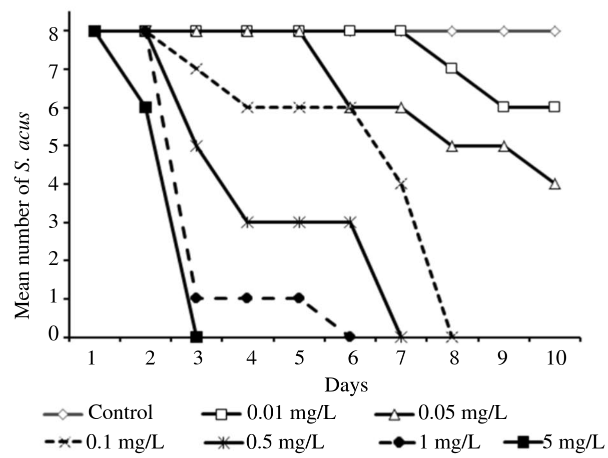

Figure 4. Mean number of *S. acus* surviving in seawater containing different copper concentrations.

4. Discussion

Cu is essential to many organisms but is potentially harmful at some levels of exposure[16,17,26-28]. The results of the present study agree with those of toxicity studies on other invertebrates[16,17,20-

23] including shrimps[18,19,26]. Bat *et al*.[19] found that non-essential metal Pb is significantly less toxic to *Palaemon adspersus* than copper, which is an essential metal. It may be owing to geography that the sites have high background Cu levels[7,29] and invertebrates may have developed either a physiological or genetic adaptation or a combination of both to some metals[8,9,30,31]. Similarly, Bat *et al.*[32] indicated that another essential metal Zn was more toxic to the polychaete worm *Hediste diversicolor* than the non-essential metal Pb.

In some cases the effects of excess metal may not be immediately apparent, if, for example, it is bound in an inert status. This kind of concealed effects will exert stress on cell function, the effects of which may only be obvious over an extended period of time, or when an organism is exposed to contaminant which may give an additional stress. The fact that metals act at the cellular level by disrupting enzymatic processes explains both why they are toxic at even very low concentrations, and the great variety of detrimental effects on different organisms. The Laizhou Bay is potentially contaminated by metals from industrial discharges and metal concentrations in shrimps *Crangon affinis* indicated that the metal pollution induced disturbances in osmotic regulation and energy metabolism and reduced anaerobiosis, lipid metabolism, and muscle movement[33].

Cu occurs naturally as metallic copper, copper sulphide and copper oxide[29,34]. In industrialized areas heavy metals including copper are an important component in waste water and the input of copper into the environment by this route may exceed the natural input by several orders of magnitude[35,36]. For example China is a major country in shrimp production and copper sulphate is usually used to eliminate filamentous algae and phytoplankton in shrimp farms[18]. Guo *et al*.[18] reported that copper treatment induced dose and time dependent toxicity to shrimp *Litopenaeus vannamei*.

In many amphipods and polychaetes, particulate-bound metal ions are the primary source of metal uptake, especially for suspension and deposit feeding animals which ingest large quantities of particulate organic material as food[37]. For benthic organisms, metal-contaminated sediment is probably a major source of metal uptake[38,39]. In these organisms metal uptake is either from contact with the contaminated sediment or from the interstitial water, which may contain high concentrations of soluble metal as a result of leaching[16].

The aim of the present study was to evaluate the effects of Cu at known concentrations on two organisms *C. crangon* and S. *acus* which have been shown to have good potential for sediment toxicity test and are likely to be used in the future studies. Survival rate of both organisms decreased with increase in the Cu concentrations in the water. Here, both *C. crangon* and *S. acus* have been shown to be a suitable test species to assess heavy metal toxicity using static 10-day and 21-day bioassays. Several toxicity testing methods have been developed since the EPA/COE testing protocol was devised[25], involving a great variety of test species[37]. However, little is known about the effects of specific contaminants on the bioassay response. The present study has confirmed the potential of both species for toxicity bioassays, both species meeting most of the criteria required[16] for suitable toxicity test organisms.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

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References

[1] Bat L, Özkan EY. Heavy metal levels in sediment of the Turkish Black Sea coast. In: Bikarska I, Raykov V, Nikolov N, editors. *Progressive engineering practices in marine resource management.* Hershey: IGI Global; 2015, p. 399-419.

[2] Bat L, Özkan EY, Öztekin HC. The contamination status of trace metals in Sinop coast of the Black Sea, Turkey. *Caspian J Environ Sci* 2015; **13**(1): 1-10.

[3] Bat L. Heavy metal pollution in the Black Sea. In: Düzgüneş E, Öztürk B, Zengin M, editors. *Turkish fisheries in the Black Sea*. Istanbul: Turkish Marine Research Foundation (TUDAV); 2014, p. 71-107.

[4] Ahmed Q, Ali QM, Bat L. Assessment of heavy metals concentration in holothurians, sediments and water samples from coastal areas of Pakistan (Northern Arabian Sea). *J Coast Life Med* 2017; **5**(5): 191-201.

[5] Bat L, Arici E, Sezgin M, Sahin F. Heavy metal levels in commercial fishes caught in the Southern Black Sea coast. *Int J Environ Geoinform* 2017; **4**(2): 94-102.

[6] EUR-Lex. [Directive 2008/56/EC of the European Parliament and of the Council of 17 June 2008 establishing a framework for community action in the field of marine environmental policy]. EUR-Lex; 2008. French. [Online] Available from: http://eur-lex.europa.eu/legal-content/FR/TXT/HTML/?uri=CELEX:32008L0056&from=EN [Accessed on 18th July, 2017]

[7] Bryan GW. Some aspects of heavy metal tolerance in aquatic organisms. In: Lockwood APM, editor. *Effects of pollutants on aquatic organisms*. Cambridge: Cambridge University Press; 1976, p. 7-34.

[8] De Nicola Gidudici M, Migliore L, Guarino SM, Gambardella C. Acute and long-term toxicity to *Idotea baltica* (Crustacea, Isopoda). *Mar Pollut Bull* 1987; **18**: 454-8.

[9] De Nicola Gidudici M, Guarino SM. Effects of chronic exposure to cadmium or copper on *Idotea baltica* (Crustacea, Isopoda). *Mar Pollut Bull* 1989; **20**: 69-73.

[10] Bilgin S, Samsun O. Fecundity and egg size of three shrimp species, *Crangon crangon*, *Palaemon adspersus* and *Palaemon elegans* (Crustacea: Decapoda: Caridea), off Sinop Peninsula (Turkey) in the Black Sea. *Turk J Zool* 2006; **30**: 413-21.

[11] Bilgin S, Ozen O, Ates AS. Spatial and temporal variation of *Palaemon adspersus*, *Palaemon elegans*, and *Crangon crangon* (Decapoda: Caridea) in the Southern Black Sea. *Estuar Coast Shelf Sci* 2008; **79**: 671-8.

[12] Bilgin S, Yılmaz N. Population dynamics of *Philocheras trispinosus* (Crangonidae) with abundance of other caridean shrimps (Crustacea: Decapoda) caught by beam trawl in the Southern Black Sea, Turkey. *J Coast Life Med* 2016; **4**(5): 358-63.

[13] Taskavak E, Gürkan S, Sever TM, Akahn S, Ozaydin O. Gut content and feeding habits of the great pipefish, *Syngnathus acus* Linnaeus, 1758, in Izmir Bay (Aegean Sea, Turkey). *Zool Middle East* 2010; **50**: 75-82.

[14] Bat L, Erdem Y, Ustao lu-Tırıl S, Yardım Ö. [*Fish systematic*]. Ankara: Nobel Yayın Dağıtım Ltd.; 2011. Turkish.

[15] Bilgin S, Gönlügür Demirci G. [The shrimps of the Sinop coasts of the Black Sea]. *Sci Eng J Fırat Univ* 2005; **17**: 143-50. Turkish.

[16] Bat L, Raffaelli D. Sediment toxicity testing: a bioassay approach using the amphipod *Corophium volutator* and the polychaete *Arenicola marina*. *J Exp Mar Biol Ecol* 1998; **226**: 217-39.

[17] Bat L, Raffaelli D, Marr IL. The accumulation of copper, zinc and cadmium by the amphipod *Corophium volutator* (Pallas). *J Exp Mar Biol Ecol* 1998; **223**(2): 167-84.

[18] Guo H, Li K, Wang W, Wang C, Shen Y. Effects of copper on hemocyte apoptosis, ROS production, and gene expression in white shrimp *Litopenaeus vannamei*. *Biol Trace Elem Res* 2017; doi: 10.1007/s12011-017-0974-6.

[19] Bat L, Bilgin S, Gündogdu A, Akbulut M, Çulha M. Individual and combined effects of copper and lead on the marine shrimp, *Palaemon adspersus* Rathke, 1837 (Decapoda: Palaemonidae). *Turk J Mar Sci* 2001; **7**: 103-17.

[20] Bat L, Sezgin M, Gündoğdu A, Çulha M. Toxicity of zinc, copper and lead to *Idotea baltica* (Crustacea, Isopoda). *Turk J Biol* 1999; **23**(4): 465-72.

[21] Bat L, Çulha M, Akbulut M, Gündogdu A, Sezgin M. Toxicity of zinc and copper to the hermit crab *Diogenes pugilator* (Roux). *Turk J Mar Sci* 1998; **4**: 39-48.

[22] Bat L, Gündogdu A, Sezgin M, Çulha M, Gönlügür G, Akbulut M. Acute toxicity of zinc, copper and lead to three species of marine organisms from Sinop Peninsula, Black Sea. *Turk J Biol* 1999; **23**(4): 537-44.

[23] Bat L, Akbulut M. Studies on sediment toxicity bioassays using *Chironomus thummi* K, 1911 larvae. *Turk J Zool* 2001; **25**: 87-93.

[24] Zar JH. *Biostatistical analysis*. 2nd ed. New Jersey: Prentice Hall, Int.; 1984.

[25] United States Environmental Protection Agency. Evaluation of dredged material proposed for ocean disposal. Washington, DC: United States Environmental Protection Agency; 1991. [Online] Available from: https://clu-in.org/download/contaminantfocus/pcb/ocean-disposal-gbook.pdf [Accessed on 18th July, 2017]

[26] Ahsanullah M, Negilski DS, Mobley MC. Toxicity of zinc, cadmium and copper to the shrimp *Callianassa australiensis*. I. Effect of individual metals. *Mar Biol* 1981; **64**: 299-304.

[27] Ahsanullah M, Mobley MC, Rankin P. Individual and combined effects of zinc, cadmium and copper on the marine amphipod *Allorchestes compressa*. *Aust J Mar Freshwater Res* 1988; **39**: 33-7.

[28] Park J, Kim S, Yoo J, Lee JS, Park JW, Jung J. Effect of salinity on acute copper and zinc toxicity to *Tigriopus japonicus*: the difference between metal ions and nanoparticles. *Mar Pollut Bull* 2014; **85**(2): 526-31.

[29] Bryan GW. Heavy metal contamination in the sea. In: Johnston R, editor. *Marine pollution*. London: London Academic Press; 1976, p. 185-302.

[30] De Nicola M, Gambardella C, Guarino SM. Interactive effects of cadmium and zinc pollution on PGI and PGM polymorphisms in *Idotea baltica*. *Mar Pollut Bull* 1992; **24**: 619-21.

[31] De Nicola M, Cardellicchio N, Gambardella C, Guarino SM, Marra C. Effects of cadmium on survival, bioaccumulation, histopathology and PGM polymorphism in the marine isopod *Idotea baltica*. In: Dallinger R, Rainbow PS, editors. *Ecotoxicology of metals im invertebrates*. Boca Raton: Lewis Publishers; 1992, p. 103-116.

[32] Bat L, Gündogdu A, Akbulut M, Çulha M, Satilmis HH. Toxicity of zinc and lead to the polychaete worm *Hediste diversicolor*. *Turk J Mar Sci* 2001; **7**: 71-84.

[33] Xu L, Ji C, Zhao J, Wu H. Metabolic responses to metal pollution in shrimp *Crangon affinis* from the sites along the Laizhou Bay in the Bohai Sea. *Mar Pollut Bull* 2016; **113**(1): 536-41.

[34] Laws EA. *Aquatic pollution*. New York: John Wiley and Sons, Inc.; 1981.

[35] Wuana RA, Okieimen FE. Heavy metals in contaminated soils: a review of sources, chemistry, risks and best available strategies for remediation. *ISRN Ecol* 2011; doi: 10.5402/2011/402647.

[36] Science Communication Unit, University of the West of England. Science for environment policy in-depth report: soil contamination: impacts on human health. Bristol: Science Communication Unit, University of the West of England; 2013. [Online] Available from: http://ec.europa.eu/environment/integration/research/newsalert/pdf/IR5_en.pdf [Accessed on 18th July, 2017]

[37] Bat L. A review of sediment toxicity bioassays using the amphipods and polychaetes. *Turk J Fish Aquat Sci* 2005; **5**: 119-39.

[38] Superville PJ, Prygiel E, Magnier A, Lesven L, Gao Y, Baeyens W, et. al. Daily variations of Zn and Pb concentrations in the Deûle River in relation to the resuspension of heavily polluted sediments. *Sci Total Environ* 2014; **470**: 600-7.

[39] Costello DM, Burton GA, Hammerschmidt CR, Rogevich EC, Schlekat CE. Nickel phase partitioning and toxicity in field-deployed sediments. *Environ Sci Technol* 2011; **45**: 5798-805.