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Acute toxicity of metals and reference toxicants to a freshwater ostracod, *Cypris subglobosa* Sowerby, 1840 and correlation to EC_{50} values of other test models

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ABSTRACT

The ostracod *Cypris subglobosa* Sowerby, 1840 static bioassay test on the basis of a 48 h of 50% of immobilization (EC_{50}) has been used to measure the toxicity of 36 metals and metalloids and 12 reference toxicants. Among the 36 metals and metalloids, osmium (Os) was found to be the most toxic in the test while boron (B), the least toxic. The EC_{50} values of this study revealed positive linear relationship with the established test models of cladoceran (*Daphnia magna*), sludge worm (*Tubifex tubifex*), chironomid larvae (*Chironomus tentans*), protozoan (*Tetrahymena pyriformis*), fathead minnow (*Pimephales promelas*), bluegill sunfish (*Lepomis macrochirus*), and aquatic macrophyte duckweed (*Lemna minor*). Correlation coefficients (r^2) for 17 physicochemical properties of metals or metal ions and EC_{50}s (as pM) were examined by linear regression analysis. The electronegativity, ionization potential, melting point, solubility product of metal sulfides (pK_{sp}), softness parameter and some other physicochemical characteristics were significantly correlated with EC_{50}s of metals to *C. subglobosa*. The reproducibility of toxicity test was determined using 12 reference toxicants. The coefficient of variability of the EC_{50}s ranged from 6.95% to 55.37% and variability was comparable to that noticed for *D. magna* and other aquatic test models. The study demonstrated the need to include crustacean ostracods in a battery of biotests to detect the presence of hazardous chemicals in soils, sewage sludges, sediments and aquatic systems.

1. Introduction

Industrial pollution in our environment has led to the development of more efficient detection of procedures aimed at the protection of human health, natural resources, terrestrial and aquatic lives. There is an increasing body of evidence to support the significant role of invertebrates in assessing impacts of environmental contaminants on freshwater ecosystems [1]. Enormous hard work has been directed to identify viable and ecologically relevant freshwater invertebrates as tools for ecotoxicological studies [2,3]. Ostracods have been of recent interest as a test model organism for environmental, paleoenvironmental and toxic stress studies [4–6]. Toxic effects of heavy metals [7,8], sewage sludges [9,10], and industrial pollutants [11–13] to freshwater ostracods with particular reference to *Cypris subglobosa* and *Heterocypris incongruens* have been studied. Recently, a new cyst-based 6-day culture/maintenance free, rapid, reliable, user-friendliness, and low-cost solid-phase direct contact microbiotest has been developed with an ostracod *H. incongruens* for toxicity monitoring of soils and river sediments in Belgium and Canada [14–17]. Efforts have also been made to evaluate the effects of soil PAH concentration and to assess the toxic effects by a battery of ecotoxicological tests using an ostracod species *H. incongruens* [18,19]. Interestingly, *C. subglobosa*, an Indian freshwater ostracod too has a number of gifted qualities, which make it a candidate worth consideration in such efforts. The wide distribution of this species in the Indian sub-continent [20], ease in handling, maintenance and culture in the laboratory; small size, and low cost of microbiotests make the *C. subglobosa* a good possible alternative option for rapid screening and monitoring of environmental pollutants in soils, sewage sludges, sediments and freshwater systems. Freshwater and marine ostracod plays important roles in aquatic food chain(s) and paleoecological studies [21].

The correlation between acute and chronic toxicity and physicochemical properties of inorganic substances is valuable in predicting toxicity to various biological important species and human health. An easy mathematical model with the use of physicochemical properties could become an important research tool for predicting the metal cations toxicity to aquatic and terrestrial biota. Thus, two types of information are required for each test metal ions

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such as the some measure of biological activity (e.g., EC_{50} value) and the second, a description of one or more physicochemical parameters. Attempts have been made to correlate and predict the metal ions toxicities to the various aquatic and terrestrial test models according to the physicochemical properties of metals or metal ions [22,23].

The freshwater ostracod *C. subglobosa* looks like seed (also known as seed shrimp) is a small crustacean typically ranges between 0.5 and 2 mm in length. They have calcareous bivalve carapace, free-swimming, benthonic in habitat, and filter feeders. Like other crustaceans, *C. subglobosa* grow by moulting (ecdysis). The first purpose of the present study was to develop baseline database concerning effects of 36 metals and metalloids and 12 reference toxicants to an ostracod, *C. subglobosa* Sowerby, 1840 which would offer an opportunity to increase both the knowledge of effects on freshwater ecosystems and the potency of this widely distributed organism as tool for ecotoxicological studies and second aim was to determine the relationship of toxicity (EC_{50}) values with the physicochemical characteristics of metals or metal ions and also to find out a correlation of EC_{50}s with the commonly established freshwater test models using the statistical procedures. The third aim of the study was to described the consistency and the sensitivity of the immobilization responses of *C. subglobosa* to each of 12 reference toxicants, which were selected as representative of chemicals commonly found in wastewater streams, including those from sewage treatment facilities, metal and mining industries, fuel refineries and fertilizer manufacturers.

2. Materials and methods

2.1. Test species

Freshwater ostracod, *C. subglobosa* Sowerby, 1840 were collected with the help of plankton net from fish ponds situated at Gheru Campus of IITR, Lucknow, India, during the year 2007–2008 and acclimatized to laboratory conditions for 3–4 days prior to experiments.

2.2. Test chemicals

All the tested metallic salts were reagent grade (>98–99.9% purity) in quality and purchased from Sigma–Aldrich, BDH, SRL (India), and E. Merck (India). Chemical formulae of the metal and metalloid salts and valences used in the present study are given in Table 1. The reference toxicants used are listed in Table 5 and purchased from SRL (India), and E. Merck (India).

2.3. Acute toxicity bioassays

A stock solution from each metal salt was prepared in double glass-distilled water. Serial dilutions were prepared from the respective stocks to the desired range; so all the concentrations referred in this paper are nominal. Final test concentrations were based on the results of preliminary range-finding short-term (48 h) tests. Ostracods were exposed for 48 h to logarithmic series of concentrations (7–10) of metals and reference toxicants. Nominal test concentrations in the range of 0.001, 0.01, 0.1, 1, 10, 100, 1000 and 10,000 $mg\,l^{-1}$ were selected for range-finding tests. Test water was renewed after 24 h and the same test concentration from stock solution after dilution was added to each concentration. Ten ostracods (*C. subglobosa*) were exposed to each test concentration in 20 ml glass petri dishes, and each concentration was tested in replicates of three. However, for reference toxicity studies, 9–12 replicates of each concentration were used to determine the replicate variability in results. Short-term static renewal range-finding and definitive tests for 48 h were performed. Test water renewed after 24 h of exposure. The test concentrations were given as $mg\,l^{-1}$ of metals and metalloids. In the definitive tests concentrations in narrow range such as 1, 3.2, 5.6, 10, 32, 56, and 100 $mg\,l^{-1}$ were selected. For pentachlorophenol, malathion, endosulfan, and lindane stocks were prepared in 50% acetone solvent. Maximum concentration of acetone used in the test was 0.2 ml in the test solution. In the control test, 0.2 ml acetone per petri dish (20 ml test solution) was used. Control experiments were run under similar conditions without the addition of toxicants.

Endpoint used in the bioassay was immobility, i.e., the inhibition to swim within 15 second after gently agitation of the test container. Immobility implies the organism usually swim helplessly at the bottom of the container and use their limbs to crawled with difficulty. The immobilized ostracods were removed and recorded after specified time of intervals 30 min, 1, 2, 4, 12,24,33 ± 3 and 48 h. The EC_{50} values were based on the nominal concentrations of added metals and reference toxicants.

2.4. Physicochemical parameters

The physicochemical properties of test water were determined by standard methods [24]. The mean and range of physicochemical characteristics of well water used in the present study were as follows: temperature 21 (20–22) °C; pH 7.6 (7.4–7.7); dissolved oxygen 5.4 (5.1–6.1) $mg\,l^{-1}$; total hardness 245 (230–250) $mg\,l^{-1}$ as $CaCO_3$ and total alkalinity 400 (390–410) $mg\,l^{-1}$ as $CaCO_3$. Mean and range of selected heavy metals ($mg\,l^{-1}$) in control test water were: Zn, 0.054 (0.041–0.65); Cu, 0.043 (0.031–0.056); Ni, 0.040 (0.032–0.068); Fe, 0.0400 (0.025–0.059); Cd, 0.006 (0.004–0.008); and Cr, 0.004 (0.001–0.031). Heavy metals were analyzed using a simultaneous multielement Atomic Absorption Spectrophotometer (PerkinElmer Analyst 300, USA). In some of the test solutions such as Ba, Fe, Ca, Pb, Zn, Sn, and Bi showed precipitate after few hours of exposure.

2.5. Experimental conditions

Living specimens were taken to the laboratory and placed in 5-l glass beaker filled with filtered well water. Test animals were acclimatized to laboratory conditions in environmental growth chamber at 21 ± 1 °C for 2–3 days. Growth chamber was equipped with two 60 W cool white fluorescent lights. For each toxicant, tests were carried under light: dark cycle of 12:12 h. Animals were fed during acclimatization period but were starved during the microbioassay tests. Food consisted of a 1000 $mg\,l^{-1}$ suspension of dry fish food (Shalimar Fish Food Co., Mumbai) and yeast. The composition of fish food was as crude protein 52%, crude fiber 7%, crude fat 8% and moisture 8%.

2.6. Data analysis

The 24 h and 48 h EC_{50} [effective concentration at which 50% immobilized response was recorded] values and their 95% confidence limits were calculated by the moving-average-angle method [25]. Metal characteristics constants of each tested metals or metal ions were collected from literature sources [26]. The constants included were electronegativity, softness parameters, atomic weight, atomic number, melting point, specific density, ionization potential, and stability constants with sulfides, etc. The 48 h EC_{50} values for the all tested metals and metalloids were determined and corresponding molarity (M) values were determined as negative log ($-\log M$) of molarity (Table 1). Statistical analysis was performed to find a general correlation, if any, between physicochemical properties and metal ion toxicity. The correlation coefficient (r) between physicochemical properties and EC_{50} values (pM) was determined by least square procedure [27]. The linear regression equations and correlation coefficients (r^2) were obtained between the 48 h EC_{50}s for *C. subglobosa* and other established aquatic toxicity test models. The acute toxicity values for water flea *Daphnia magna*, protozoan, *Tetrahymena pyriformis*, sludge worm, *Tubifex tubifex*, chironomid larvae, *Chironomus tentans*, fathead minnow, *Pimephales promelas*, bluegill sunfish, *Lepomis macrochirus*, and duckweed, *Lemna minor* were collected from the published literature [28–33]. For *D. magna* 48 h EC_{50}s were obtained from the earlier published literature [2,34]. Mean EC_{50}s, the standard deviation and coefficient of variation (CV = standard deviation/mean × 100%) were calculated for each reference toxicant and these values were used to determine the variability and precision of each tested reference toxicant. Replications of 9–12 tests were carried out for reference chemicals.

3. Results

3.1. Metals and metalloids toxicities and interspecies correlation

In control tests, ostracods remain active throughout the test period. The EC_{50}s and their 95% confidence limits of metals and metalloids were arranged in decreasing rank order of toxicity (Table 1). The results indicate that Os, Ag, Pt, and Hg were the most toxic metals, while B, Mg, Ba, and Sb were the least toxic among the 36 tested metals and metalloid salts. The 48 h EC_{50}s for Os, Ag, Pt, Hg, and Cu for *C. subglobosa* calculated were 0.007, 0.013, 0.095, 0.097, and 0.55 mg l^{-1}, respectively. The 48 h EC_{50} values suggested the decreasing rank order toxicity of metals and metalloids was as follows: Os > Ag > Pt > Hg > Cu > Pd > Cd > Li > Zn> As > Be > Cr^{3+} > U > Mn > Se > Cr^{6+} > Co> Bi > Pb > Mo > Sn > La > Al > Zr > Te > Ni > Fe > W > Sr > K > Na >Ca> Sb > Ba > Mg > B (Table 1).

The sensitivity comparison between *D. magna* and *C. subglobosa* standard acute toxicity tests were based on 48 h EC_{50} data obtained for 28 metals. The toxicity values were obtained from the earlier published literature [2,29–34] (Table 2). To clarify the sensitivity of *C. subglobosa* species, the acute toxicity data were compared to that of *D. magna* by statistical analysis using their EC_{50} (pM) values for 48 h of exposure time (Fig. 1a). The coefficient of correlation ($r^2 = 0.872$, $p \leq 0.01$, $n = 28$) and spearman rank correlation (rs = 0.893) suggested that interrelationship with the acute toxicity of metals showed high degree of linear correlation. The relationship between the toxicity data for the metals tested in this study on *C. subglobosa* was also compared with corresponding data for *T. tubifex* (Fig. 1b; $r^2 = 0.695$; $p \leq 0.01$), *C. tentans* (Fig. 1c; $r^2 = 0.878$; $p \leq 0.01$), *T. pyriformis* (Fig. 1d; $r^2 = 0.453$, $p \leq 0.05$), fishes *P. promelas* (Fig. 2a; $r^2 = 0.795$, $p \leq 0.01$), *L. macrochirus* (Fig. 2b; $r^2 = 0.956$, $p \leq 0.01$). The EC_{50} values determined using 11 metals of *C. subglobosa* and aquatic macrophyte duckweed (*L. minor*) were positively correlated (Fig. 2c; r^2 = 0.424, $p \leq 0.05$). In the present study an attempt was made to determine a relationship among the acute toxicities of metals to freshwater fish, *L. macrochirus* and *P. promelas* (96 h LC_{50}s) and *C. subglobosa* (48 h EC_{50}s). A highly significant ($r^2 = 0.795–0.956$, $p \leq 0.01$) correlation was found. Water flea *D. magna* were generally being slightly more sensitive to acute toxicity of the transitional metals. The relationship between these two species was as follows: pM EC_{50} *D. magna* = (pM *C. subglobosa* EC_{50} × 0.945) + 0. 608 (Table 3). The correlation coefficient (r) for *D. magna*, *L. macrochirus*, *C. tentans*, were greater than 0.85 and for *P. promelas*, *T. tubifex*, and for *T. pyriformis* it was greater than 0.453. The regressions analyzed show *C. subglobosa* are better surrogates for the other aquatic animals tested.

Table 1
Acute toxicity of various metal and metalloid ions to a freshwater ostracod, *Cypris subglobosa*.

Salt used	Metal ions	EC_{50} value and 95% confidence limits (mg l^{-1}) 48 h		Molarity, *M*	−log *M* (pM)
OsO_4	Os^{4+}	0.011 (0.008–0.021)	0.007 (0.005–0.013)	3.68×10^{-8}	7.43
$AgNO_3$	Ag^{1+}	0.037 (0.032–0.042)	0.013 (0.0117–0.0154)	1.21×10^{-7}	6.91
$PtCl_2$	Pt^{4+}	0.114 (0.085–0.187)	0.095 (0.072–0.119)	4.86×10^{-7}	6.31
$HgCl_2$	Hg^{2+}	0.369 (0.322–0.419)	0.097 (0.085–0.113)	4.84×10^{-7}	6.31
$CuSO_4{\cdot}5H_2O$	Cu^{2+}	3.42 (2.67–4.38)	0.55 (1.27–1.89)	8.65×10^{-6}	5.06
PdCl	Pd^{4+}	0.351 (0.214–0.525)	0.195 (0.127–0.287)	1.83×10^{-6}	5.74
$CdCl_2{\cdot}6H_2O$	Cd^{2+}	3.22 (2.63–4.11)	0.821 (0.729–0.992)	7.30×10^{-6}	5.13
$LiSO_4$	Li^{1+}	2.13 (1.79–2.44)	46.85 (39.49–57.36)	2.52×10^{-4}	3.60
$ZnSO_4{\cdot}7H_2O$	Zn^{2+}	3.40 (2.78–4.02)	85.04 (71.37–105.90)	4.63×10^{-5}	4.33
Na_3AsO_3	As^{3+}	8.85 (7.74–10.27)	5.03 (4.40–6.02)	6.71×10^{-5}	4.17
$BeSO_4$	Be^{2+}	20.46 (16.43–24.36)	8.05 (6.69–8.73)	8.93×10^{-4}	3.04
K_2CrO_4	Cr^{6+}	20.86 (17.48–24.40)	8.75 (7.16–11.30)	1.68×10^{-4}	3.77
$UO_2(CH_3COO)_2{\cdot}2H_2O$	${UO_2}^{2+}$	62.72 (56.37–73.98)	10.41 (7.52–13.80)	4.77×10^{-5}	4.36
$MnSO_4{\cdot}2H_2O$	Mn^{2+}	15.83 (13.36–19.46)	11.77 (9.57–13.96)	2.41×10^{-4}	3.66
$Na_2Se_2O_3$	Se^{2+}	51.52 (45.33–59.45)	14.92 (12.41–17.66)	1.68×10^{-4}	3.77
$K_2Cr_2O_7$	Cr^{3+}	37.72 (32.62–42.65)	2 5.57 (22.71–29.64)	3.18×10^{-4}	3.72
$(CH_3COO)_2Co{\cdot}4H_2O$	Co^{2+}	25.49 (17.55–34.80)	27.82 (24.75–32.52)	4.72×10^{-4}	3.32
$Bi(NO_3)_3{\cdot}5H_2O$	Bi^{3+}	46.85 (39.49–57.36)	37.08 (24.22–50.17)	1.77×10^{-4}	3.75
$Pb(CH_3COO)_2{\cdot}4H_2O$	Pb^{2+}	46.85 (39.49–57.36)	40.19 (34.13–46.87)	1.94×10^{-4}	3.71
$Na_2MoO_4{\cdot}2H_2O$	Mo^{2+}	85.04 (71.37–105.90)	40.19 (34.13–46.87)	1.94×10^{-4}	3.71
$La(OH)_3$	La^{3+}	50.75 (39.44–63.13)	41.46 (33.05–50.37)	4.32×10^{-4}	3.71
$SnCl_2{\cdot}2H_2O$	Sn^{2+}	56[a]	50.75 (39.44–63.11)	3.65×10^{-4}	3.43
$NiCl_2{\cdot}6H_2O$	Ni^{2+}	86. 84 (77.21–118.3)	75.78 (65.79–89.08)	1.29×10^{-3}	2.88
$Al(NH_3SO_4)_2{\cdot}12H_2O$	Al^{3+}	108.9 (87.47–131.70)	100.90 (75.06–131.61)	3.70×10^{-3}	2.42
K_2TeO_3	Te^{2+}	278.2 (240.8–357.6)	106.4 (81.81–139.7)	8.33×10^{-4}	3.07
$ZrOCl_2$	Zr^{2+}	134.2 (113.7–158.3)	114.0 (91.62–136.2)	1.25×10^{-3}	2.90
$FeCl_3{\cdot}6H_2O$	Fe^{3+}	222.7 (193.6–252.2	115.2 (98.51–130.0)	2.10×10^{-3}	2.64
$Na_2WO_4{\cdot}2H_2O$	W^{6+}	227.6 (168.5–266.5)	144.6 (120.3–178.2)	7.85×10^{-4}	3.10
$SrCl_2{\cdot}6H_2O$	Sr^{2+}	263.2 (214.7–337.1)	180[a]	2.05×10^{-3}	2.68
KCl	K^{1+}	674.3 (564–787)	362 (293–430)	9.25×10^{-3}	2.03
$CaCl_2$	Ca^{2+}	674.2 (563–787)	512 (430–634)	1.27×10^{-2}	1.89
NaCl	Na^{1+}	885.3 (780 1050)	519.6 (446–628)	2.26×10^{-2}	1.65
Sb_2O_3	Sb^{3+}	709 (599–832)	560[a]	4.56×10^{-3}	2.33
$BaSO_4$	Ba^{2+}	798 (615–985)	634 (574–685)	4.61×10^{-3}	2.34
$MgSO_4{\cdot}7H_2O$	Mg^{2+}	704 (640–778)	704 (640–778)	2.89×10^{-2}	1.53
$Na_2B_4O_7{\cdot}10H_2O$	B^{3+}	2400 (2041–2736)	1645 (1332–2135)	0.15×10^{-1}	0.81

[a] 95% confidence intervals cannot be calculated.

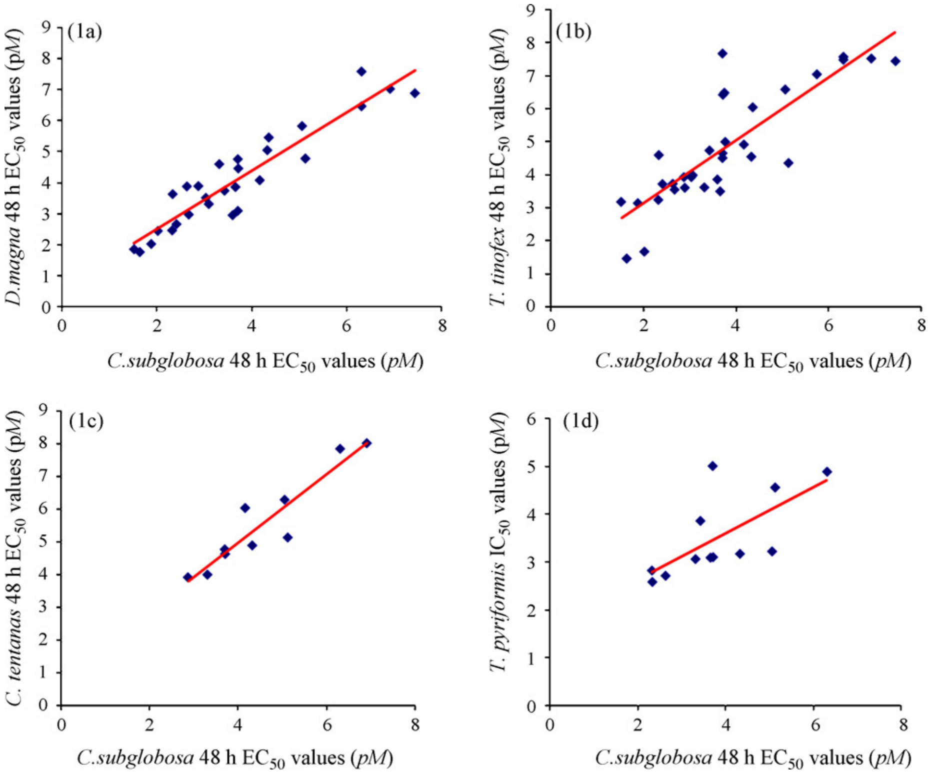

Fig. 1. Correlation between the $-\log M$ (pM) EC$_{50}$ values of *C. subglobosa* and $-\log M$ (pM) EC$_{50}$ values of other test models exposed to metals and metalloids, described by the least squares solid line: (a) for *Daphnia magna*, $y = 0.945x + 0.608$, with $r^2 = 0.872$, spearman rank correlation (rs) = 0.893, $n = 28$, $p \leq 0.01$; (b) for *Tubifex tubifex*, $y = 0.953x + 1.226$, with $r^2 = 0.695$, spearman rank correlation (rs) = 0.850, $n = 33$, $p \leq 0.01$; (c) for *Chironomus tentans*, $y = 1.05x + 0.769$, with $r^2 = 0.878$, spearman rank correlation (rs) = 0.927, $n = 10$, $p \leq 0.01$; and (d) for *Tetrahymmna pyriformis*, $y = 0.471 + 1.677$, with $r^2 = 0.453$, spearman rank correlation (rs) = 0.783, $n = 12$, $p \leq 0.01$.

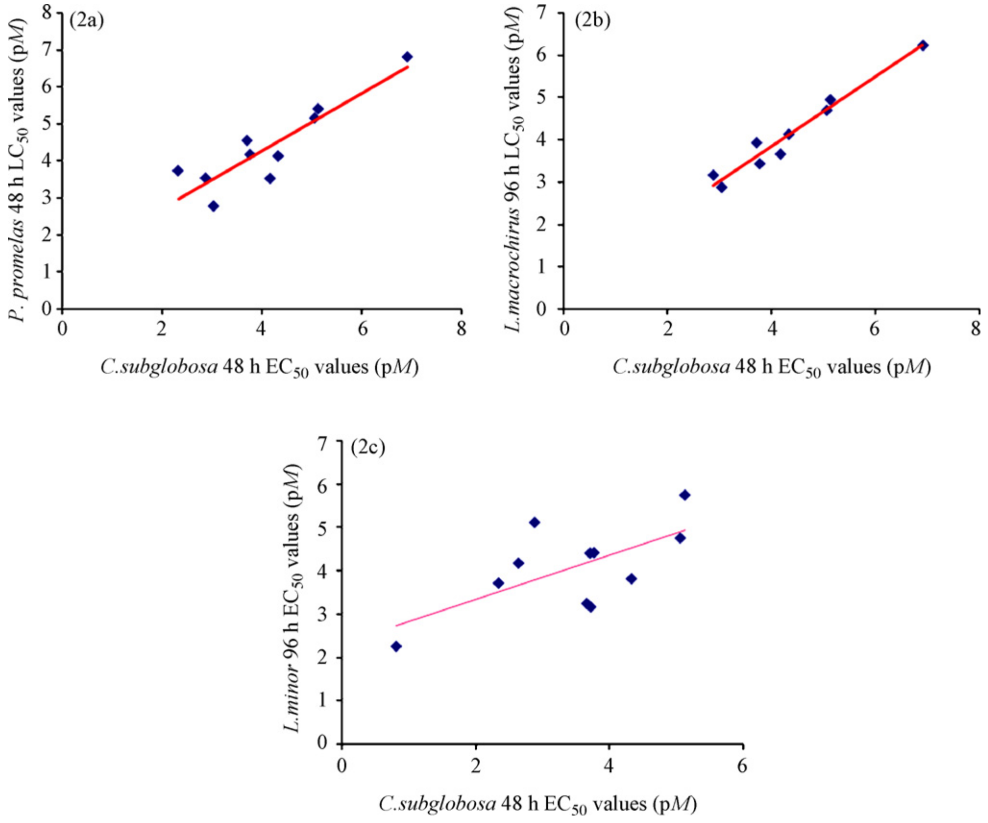

Fig. 2. (a–c) Correlation between the $-\log M$ (pM) EC$_{50}$ values of *C. subglobosa* and $-\log M$ (pM) EC$_{50}$ values of fishes and duckweed exposed to metals, described by the least squares solid line: (a) for *Pimephales promelas*, $y = 0.781x + 1.160$, with $r^2 = 0.795$, spearman rank correlation (rs) = 0.933, $n = 10$, $p \leq 0.01$; (b) for *Lepomis macrochirus*, $y = 0.823x + 0.562$, with $r^2 = 0.956$, spearman rank correlation (rs) = 0.927, $n = 9$, $p \leq 0.01$; and (c) for *Lemna minor*, $y = 0.509x + 2.316$, with $r^2 = 0.424$, spearman rank correlation (rs) = 0.564, $n = 11$, $p \leq 0.05$.

Table 2
Acute toxicity (LC_{50} or EC_{50}) values of selected metals and metalloids used for the comparison of species sensitivities.

Metal used	EC_{50} or LC_{50} or IC_{50} (mg l^{-1})							
	C. subglobosa[a]	*D. Magna*[b,c]	*T. tubifex*[d]	*C. tentans*[e]	*T. Pyriformis*[f]	*P. promelas*[g]	*L. macrochirus*[g]	*L. minor*[h]
Os	0.007	0.024	0.007	–	–	–	–	–
Ag	0.013	0.014	0.03	0.01	–	–	–	–
Pt	0.095	0.066	0.06	–	–	–	–	–
Hg	0.097	0.022	0.05	0.03	2.5	–	–	–
Cu	0.055	0.014	0.16	0.33	30	0.427	1.23	1.1
Pd	0.195	–	0.09	–	–	–	–	–
Cd	0.82	8.07	47.53	8.05	3.0	0.43	1.23	0.2
Li	46.85	7.82	9.34	–	–	–	–	–
Zn	85.04	0.13	17.87	8.20	43	4.7	4.7	10
As	5.03	74.0	8.87	0.68	–	15.7	16.2	–
Be	8.05	2.81	10.25	–	–	0.2	1.3	–
Cr^{6+}	8.75	1.84	0.19	11.80	40	–	–	35
U	10.41	0.83	2.05	–	–	–	–	–
Mn	11.77	10.27	170.61	–	44	–	–	31
Se	14.92	–	7.71	–	–	–	–	2.4
Co	27.82	1.67	139.32	56.87	50	–	–	–
Bi	37.08	–	0.66	–	–	–	–	–
Pb	40.19	8.69	0.04	34.67	180	5.7	23.8	8
Mo	40.19	78.08	28.91	–	–	–	–	–
La	41.46	–	29.38	–	–	–	–	–
Sn	50.75	6.84	21.23	–	40	–	–	–
Ni	75.78	5.70	66.75	69.50	–	17	40	0.45
Al	100.90	59.6	50.23	–	–	–	–	–
Te	106.40	–	125.60	–	–	–	–	–
Zr	114	–	221.18	–	–	–	–	–
Fe	115.20	2.92	101.18	–	106	–	–	3.7
W	144.60	65.18	–	–	–	–	–	–
Sr	180	94	240.8	–	–	–	–	–
K	362	160.45	812.8	–	–	–	–	–
Ca	512	560	281.19	–	–	–	–	–
Na	519.6	423.13	781	–	–	–	–	–
Sb	560	423.45	678	–	16	22	–	–
Ba	634	14.50	33.65	–	530	–	–	26
Mg	704	288.72	158.13	–	–	–	–	–
B	1645	0.4	–	–	–	–	–	>60

[a] Data collected from present study.
[b] Data collected from Khangarot and Ray [2].
[c] Data collected from Rathore [34].
[d] Data collected from Khangarot [30].
[e] Data collected from Khangarot and Ray [29].
[f] Data collected from Sauvant et al. [32].
[g] Data collected from LeBlanc [31].
[h] Data collected from Wang [33].

3.2. Correlation of EC_{50}s with physicochemical properties of metal ions

The correlation coefficient values (Table 4) ranged from $r = 0.044$ (no correlation) to $r = 0.903$ (very high correlation). The excellent correlation was found only for pK_{sp} and softness parameter; there is different value for others. No significant correlations between EC_{50}s and ionic and covalent radius, boiling point and thermal conductivity were noticed. The solubility product of metal sulfide constant (pK_{sp}) closely correlated with EC_{50}s of *C. subglobosa*. No significant correlations between EC_{50}s and ionic and covalent radius, boiling point and thermal conductivity were noticed.

3.3. Reference toxicants

The assay was most sensitive to insecticides with special reference to malathion (mean 48 h EC_{50}s ranging from 0.00042 to 0.00085 mg l^{-1} in nine replicate tests). In most of the cases, increase in the exposure period; significantly increased the sensitivity of reference pollutants. The variability of *C. subglobosa* acute responses was measured by the coefficient of variation around EC_{50}s to each of toxicant ranges from 6.95% to 55.37%. Coefficient of variation for sodium fluoride ranged from 11.04% to 30.11% for 24 h and 48 h EC_{50}, respectively. Acute toxicity responses for *C. subglobosa* exhibited the greatest variation when exposed to sodium lauryl sulfate and vari-

Table 3
Correlation between the acute sensitivities of ostracod (*C. subglobosa*) 48 h EC50 values (pM) with other established aquatic test models to metals and metalloids.

Test model	No. of metals used	Regression equation	Correlation coefficient (r^2)	Rank correlation (rs)	Statistical significance
D. magna	28	$y = 0.945x + 0.608$	0.872	0.893	$p \leq 0.01$
T. tubifex	33	$y = 0.953x + 1.226$	0.695	0.850	$p \leq 0.01$
C.tentans	10	$y = 1.052x + 0.769$	0.878	0.927	$p \leq 0.01$
T. pyriformis	12	$y = 0.479x + 1.677$	0.453	0.783	$p \leq 0.01$
P. promelas	10	$y = 0.781x + 1.160$	0.795	0.933	$p \leq 0.01$
L. macrochirus	9	$y = 0.823x + 0.562$	0.956	0.927	$p \leq 0.01$
L. minor	11	$y = 0.509x + 2.316$	0.424	0. 564	$p \leq 0.05$

Table 4
Correlation of *C. subglobosa* EC50 values (pM) with selected physicochemical properties of metals or metal ions at 48 h of exposure.

Physicochemical properties	No. of metals used in equation	Correlation coefficient	Statistical significance
Atomic number	36	0.529	$p \le 0.005$
Atomic weight	36	0.563	$p \le 0.005$
Specific density	36	0.737	$p \le 0.005$
Melting point (°C)	36	0.238	NS
Boiling point (°C)	36	0.175	NS
Thermal conductivity	36	0.107	NS
Electrical resistance	36	0.288	NS
Ionization potential	36	0.425	$p \le 0.005$
Electronegativity	36	0.501	$p \le 0.005$
Atomic electron affinity	36	0.485	NS
Ionic radius	36	0.044	NS
Covalent radius	36	−0.124	NS
Atomic radius	36	−0.114	NS
Heat capacity	36	−0.305	NS
Softness parameter (σ_P)	19	−0.808	$p \le 0.005$
pK_{sp} values for metal sulfide	8	0.903	$p \le 0.005$
pK_{sp} values for EDTA	19	0.233	NS

ability increased with increasing exposure period. The coefficient of variation ranged from 13.47% for the 24 h exposure to 42.32% for the 48 h exposure. Acute toxicity results of pentachlorophenol were more consistent appeared to decrease with increasing exposure period (CVs of 15.65% and 12.07% for 24 h and 48 h, respectively, Table 5). In the present study, coefficient of variation was less than that of 35% for 9 reference chemicals out of 12 chemicals tested. The sensitivity of the *C. subglobosa* assay to 12 reference pollutants was similar to that reported for a freshwater cladoceran *D. magna* (see Table 5, Fig. 3).

4. Discussion

4.1. Metals and metalloids

Few Investigators reported the acute toxicity of metals to ostracods. Khangarot and Ray [7] observed the acute Cu toxicity to a freshwater ostracod (*C. subglobosa*) at different pH exposure. Toxicity of Cu increases as pH of the test medium decreases from 8.5 to 5.5 and vice versa. The 48 h EC_{50}s for *C. subglobosa* were 0.35 and 5.1 mg l^{-1} of Cu at pH 5.5 and 8.5, respectively. In the present study, pH ranges from 7.4 to 7.7. Ruiz et al. [21] reviewed the literature on the ostracod responses to pollution-induced environmental changes by anthropogenic impacts and concluded that these microorganisms can be included in the most promising sentinel groups for pollution studies because these microcrustacean showed high sensitivity to heavy metal pollution, oil-discharges and anoxic conditions. Recently, the acute (LC_{50} and EC_{50} endpoints) toxicity of 468 organic pollutants of freshwater and marine pollutants to planktonic crustaceans including eight ostracod species was determined [13]. Furthermore, ostracods were equally sensitive to pesticides [34], polycyclic aromatic hydrocarbons [16,35] and in sediment toxicity assays [15]. The present results showed that ostracod bioassay was capable of detecting the toxicity of metals and reference toxicants at parts per million (mg l^{-1}) or parts per billion (μg l^{-1}) levels. The toxic effects of metals and reference toxicants in water and wastewater should be further assessed under the different environmental, physical, chemical and biological factors. Thus, ostracod toxicity test method required further standardization. Based on its sensitivity and reliability in bioassay studies the ostracod *C. subglobosa* is proposed to be included in a battery of microbiotests to assess the risk of pollutants in water systems.

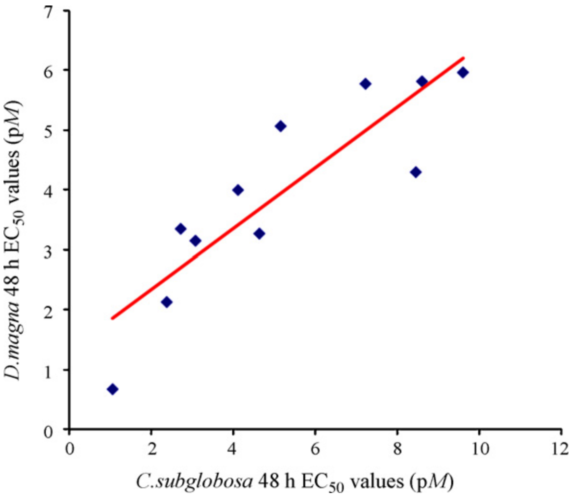

Fig. 3. Correlation between the −log *M* (pM) EC_{50} values of *C. subglobosa* and −log *M* (pM) EC_{50} values of *D. magna* exposed to reference toxicants, described by the least squares solid line: $y = 0.507x + 1.326$, with $r^2 = 0.76$, spearman rank correlation (rs) = 0.927, $n = 11$, $p \le 0.01$.

4.2. Interspecies correlation in toxicity values

A few interspecific toxicological relationships have been investigated. Comparative acute toxicity of environmental pollutants including metals on water flea (*D. magna*), bluegill (*L. macrochirus*) and fathead minnows (*P. promelas*), rainbow trout (*Salmo gairdneri*) and saltwater mysidacean (*Mysidosis bahia*), channel catfish (*Ictalurus punctataus*) were studied [31–33]. In these test models consistent trends in susceptibilities were determined. The coefficient of correlation of EC_{50}s of *D. magna* for metals and metalloids were determined and compared statistically with the tubificid worm (*T. tubifex*), fish and amphibian tadpoles and results showed the good linear correlations [34]. The sensitivity of *D. magna* as surrogate species in standard testing protocols has been confirmed by the high degree of correlation between the data for this species and that of other test models used in toxicity testing [22,23]. This work confirms our earlier results on smaller data sets that demonstrated the higher degree of relationship of the *D. magna* and fish *S. gairdneri* [28]. The toxicity of heavy metals to aquatic organisms largely varied with the water hardness and influenced the interspecies correlation of toxicity values [36]. Protozoan *T. pyriformis* regression analysis was performed for IC_{50}s described for this species and EC_{50}s for *C. subglobosa* using 12 metals [32]. This analysis was calculated to determined if lower group of invertebrate

Table 5
Mean, standard deviation and range of EC_{50} values to *C. subglobosa* and their coefficient of variable for EC_{50} values after 24 h and 48 h of exposure to 12 reference toxicants.

Reference toxicants	No. of replicates	Mean EC_{50} ± S.D. (mg l^{-1}) values (ranges)		Coefficient of variance (CV%)	
		24 h (range)	48 h (range)	24 h	48 h
Endosulfan	10	0.0021 ± 0.0004 (0.0012–0.0034)	0.001 ± 0.00032 (0.00052–0.0016)	19.76	31.01
Lindane	10	0.0016 ± 0.0004 (0.0012–0.0027)	0.001 ± 0.0002 (0.00047–0.0015)	23.71	20.43
Malathion	9	0.0087 ± 0.0035 (0.0065–0.015)	0.0068 ± 0.0014 (0.00042–0.00085)	55.37	21.22
Pentachlorophenol	10	0.027 ± 0.042 (0.195–0.035)	0.016 ± 0.037 (0.013–0.018)	15.65	12.07
Ammonium chloride	12	0.268 ± 0.08 (0.179–0.403)	0.10 ± 0.017 (0.075–0.126)	30.24	16.72
Chloroform	10	4.325 ± 0.69 (3.34–5.21)	2.803 ± 0.19 (2.57–3.06)	15.92	6.95
Sodium lauryl sulfate	12	3.90 ± 0.53 (2.64–4.88)	2.05 ± 0.87 (1.11–3.70)	13.47	42.32
Phenol	10	7.08 ± 1.23 (5.15–10.05)	6.57 ± 1.75 (4.05–8.52)	26.67	17.42
Xylene	10	213.83 ± 18.91 (100–263.8)	90 ± 35.88 (69–169)	8.84	39.87
Carbon tetrachloride	10	300.67 ± 31.42 (250.4–321.4)	180.54 ± 63.41 (102.8–355.7)	10.42	35.12
Sodium fluoride	10	290.1 ± 32.03 (241.8–349.6)	179.4 ± 30.11 (134–214)	11.04	30.11
Ethyl alcohol	9	3539 ± 349 (3141–4143)	1074 ± 169 (844–1308)	9.74	15.76

respond similarly to acute toxicity of metals. The toxicity baseline database on ostracod is still deficient. The present data clearly demonstrated that the sensitivities of aquatic specimen occupying different tropic levels in freshwater environment to the acute toxicity of metals are remarkable similar expect for *T. pyriformis*. Therefore, the acute toxicity data determined for *C. subglobosa*, can be used when estimating the hazard associated with a pollutant to other aquatic organisms. The risk of many agricultural and industrial chemicals draining into aquatic environment cannot be assessed properly until more toxicity data on these toxic substances are tested on commonly available ostracod species. The question has been raised constantly as to whether a zooplankton bioassay is comparable to a fish assay. In a chronic sensitivity study on *D. magna* and *P. promelas* a good correlation was found for a variety of chemicals [37]. The database is sufficient for *D. magna* as compared to other zooplanktons. The significant correlation of the present data with other established test models including fish indicate that ostracod bioassay is a very promising tool for acute toxicity testing. The strength of the ostracod is simple, inexpensive and sensitive. It required no special skill, training and equipments. Ostracod *C. subglobosa* can be used for screening or monitoring aquatic toxicity. If there is any indication of toxicity, further test can be followed by *D. magna* assay. The sensitivity and complementarily of the observed bioassays would be in favor of its incorporation to a 'battery' of tests used for ecotoxicological screening of environmental xenobiotics. The comparison with other test models acute toxicity values indicate that the ostracod test may be an efficient and sensitive indicator of acute aquatic toxicity for metals and other priority pollutants.

4.3. Correlation with physicochemical properties of metal ions

Relative metal acute toxicity was also predicted with least square linear regression and several metal ion characteristics for the free-living soil nematode *Caenorhabditis elegans* in an aqueous medium [38]. Recently, the relationship between 20 physical and chemical properties of metal cations and toxicity has been discussed and examined in the previous study [22]. The high correlation between toxicity values (as pM) and in the electronegativity and equilibrium constants of metal sulfides tend to support the concepts that toxicity of metal ions is a function of their electron-attracting properties and toxic action is related to their strength of covalent binding with electron-rich functional groups of the cell such as sulfhydryl or carboxyl groups.

The present data showed good correlation between the EC_{50} values for *C. subglobosa* and softness parameter (σ_P). The softness parameter (σ_P) can be defined in terms of the coordinate bond energies of the metal fluoride and metal iodide [39]. Thus, softness involves not only the metal itself, but also something of its chemistry as manifested through its bonding with two halogen ligands. In the present study, it was noticed that smaller the value of softness parameter (σ_P), higher the toxicity of metal ions. The toxicity of the most of the divalent ions was positively correlated to the softness values. The better correlation ($r^2 = 0.8$ or higher) between the acute toxicity LD_{50} of 24 metal ions in mice and *Drosophila melanogaster* with the softness parameter was noticed [40]. The physicochemical concepts relating hard/soft character of metal ion biochemistry and toxicity were discussed elsewhere [41]. There is a need, to examine the interspecies correlation in toxicity values with several other

Table 6
Acute toxicity values (48 hr EC_{50}s) for *C. subglobosa* and *D. magna* for 11 reference toxicants.

Toxicant	Ostracod *C. subglobosa* values			Water flea *D. magna* values		
	C. subglobosa 48 h EC_{50} (mg l^{-1})	48 h EC_{50} molarity, *M*	−log *M* (pM)	*D. magna* 48 h EC_{50} (mg l^{-1})	48 h EC_{50} molarity, *M*	−log *M* (pM)
Sodium fluoride	179.4	4.27×10^{-3}	2.37	307.7[b]	7.33×10^{-3}	2.13
Sodium lauryl sulfate	2.05	7.11×10^{-6}	5.15	2.44[e]	8.46×10^{-6}	5.07
Phenol	6.57	777×10^{-5}	4.11	9.13[b]	1.0×10^{-4}	4.0
Carbon tetrachloride	300.67	1.95×10^{-3}	2.71	69.37[a]	4.51×10^{-4}	3.35
Chloroform	2.803	2.35×10^{-5}	4.63	64.23[a]	5.38×10^{-4}	3.27
Ethyl alcohol	4074	8.84×10^{-2}	1.05	9788[a]	2.12×10^{-1}	0.67
Malathion	0.00068	2.06×10^{-10}	9.69	0.35[b]	1.07×10^{-7}	5.97
Pentachlorophenol	0.016	6.06×10^{-8}	7.22	0.44[a]	1.65×10^{-6}	5.78
Endosulfan	0.001	2.54×10^{-9}	8.60	0.62[c]	1.52×10^{-6}	5.82
Xylene	90	8.48×10^{-4}	3.07	75.49[d]	7.11×10^{-4}	3.15
Lindane	0.001	3.54×10^{-9}	8.45	14.50[b]	4.99×10^{-5}	4.30

[a] Khangarot and Ray [51].
[b] Lilius et al. [47].
[c] Fernandez et al. [48].
[d] Lilius et al. [49].
[e] Harrnon et al. [50].

organic and inorganic compounds, and industrial effluents reaching to the aquatic ecosystem under different environmental conditions.

4.4. Reference toxicants

The acute toxicity of 12 priority pollutants measured in the present study exhibit 10 % to 35% variability in most of the cases. The present study showed that acute responses of *C. subglobosa* were sensitive, consistent and exhibits variability acceptable for bioassays used in a biomonitoring role [42]. The Biomonitoring Science Advisory Board (BSAB) of the state of Washington considers the repeatability of the assays to be good, if coefficients of variation around EC_{50}s were less than 35% [43]. Thus, our study suggested that the degree of variability in the *C. subglobosa* acute toxicity assay is excellent and variability compares well with that noticed for other aquatic invertebrates and algal acute toxicity tests conducted elsewhere [44] (Tables 5 and 6).

The sludge toxicity is mainly due to presence of toxic heavy metals (Cd, Cu, Cr, Pb, Ni, and Zn) and organic pollutants (polycyclic aromatic hydrocarbons). Therefore, our study showed the necessity to supplement chemical studies with biological assays. An ostracod *H. incongruens* has been successfully used in a battery of microbiotest to assess the toxicity of soil PAHs in sewage sludges [8–10]. The results demonstrate that use of test batteries employing various organisms of different biological complexity of general toxicity of xenobiotics in risk assessment studies. Some researchers were already using battery of bioassays using different key species in the tropic food chains to evaluate the toxicity of environmental pollutants [45]. In 1998, a battery of six standard ecotoxicity tests including freshwater crustaceans was proposed by the French Ministry of Environment in order to assess the hazardous wastes [46]

5. Summary and conclusions

Freshwater ostracods such as *C. subglobosa* are widely distributed zooplankton in Indian sub-continent and elsewhere and play an important role in the aquatic food chain(s). The significance of a zooplankton bioassay is that they are an essential part of an aquatic ecosystem, and as such zooplankton should be protected in order to achieve a healthy environment. The results indicate that ostracod *C. subglobosa* has sensitivity quite similar to the commonly used established test models. There is a need, to examine the interspecies correlation in toxicity value with several other organic and inorganic compounds, and industrial effluents reaching to the aquatic ecosystem under different environmental conditions. The present toxicity test results have revealed that *C. subglobosa* immobilization assay was highly sensitive, easy to conduct, cost-effective, trustworthy and accurately measure the acute toxicity of metals, metalloids and reference toxicants. Thus, the *C. subglobosa* seems to be an attractive alternative bioassay procedure for routine monitoring of polluted water, industrial effluents safety evaluation, soil, and sediment toxicity studies. The results of toxicity test are useful for developing the water quality criteria for improving the human health and for other diverse uses of our Nation's Water. The invention of a large toxicity data with a fairly new test organism will certainly increase both the understanding of effects in ecosystems and the strength of microbiotest with ostracod crustacean *C. subglobosa* as test model for aquatic ecotoxicological studies.

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