atmospheric CO2 remains a global concern due to its pervasive and irreversible consequences on ecological timescales (Council, 2011). Rising atmospheric CO2 remains a global concern due to its pervasive and irreversible consequences on ecological timescales (Council, 2011). Environmental factors like ocean acidification, rainfall patterns, the rising water temperature will considerably be affected by GCC according to IPCC forecast (Hoegh-Guldberg et al., 2014). Although the interactive effects of GCC with other anthropogenic stressors such as chemical contaminants are still not fully understood, there are concerns about the influential role GCC plays on the environmental concentration of contaminants (Gouin et al., 2013), as GCC is anticipated to have a significant effect on exposure, behaviour, fate, and release of toxicants (Noyes et al., 2009).

Ocean acidification is a key threat to marine biodiversity in estuarine and coastal ecosystems (Campbell et al., 2014; Ivanna and Sokolova, 2015) and is broadly considered to represent a significant threat to global marine biodiversity (Campbell et al., 2014). In the course of the past 650 000 years preceding the industrial revolution, concentrations of atmospheric CO2 ranged from 280 ppm (Gao et al., 2019). As a result, anthropogenic activities, mainly due to the burning of fossil fuel, industrialization and changes in land use (Hooper et al., 2013), the atmospheric concentration of CO2 has increased to about 409 ppm in 2018 and is currently increasing at a rate of ~0.5 % annually (Gao et al., 2019), approximately 100 times more rapid than any change in the past 650 000 years. The net effect of increasing the partial pressure of CO2 (pCO2) or hypercapnia of seawater leads to the shift in the pH and the carbonate chemistry (Ivanina and Sokolova, 2015). The inorganic carbon system is an important chemical equilibrium in seawater chemistry and is generally responsible for controlling the seawater pH (Fabry et al., 2008). Dissolved inorganic carbon (DIC) exists in seawater in three main forms; bicarbonate ion (HCO3⁻), carbonate ion (CO3²⁻), and aqueous carbon dioxide (CO2 (aq)) which also includes carbonic acid (H2CO3). About 88 % of the carbon forms HCO3⁻ at the pH of 8.2, while 11 % is in the form of CO3²⁻, and only about 1 % of the carbon forms dissolved CO2. Carbon dioxide dissolves in seawater to form H2CO3, and most of the H2CO3...
quickly dissociates into a hydrogen ion (H+) and (HCO₃⁻). The hydrogen ion then proceeds to react with CO₃²⁻ to form bicarbonate.

Therefore, the resultant effect of seawater absorbing CO₂ is increasing concentrations of H₂CO₃, HCO₃⁻, and H⁺, and the concomitant decrease in the concentration of CO₃²⁻ and lower pH (pH = −log[H⁺]). These reactions are fully reversible, and the basic thermodynamics of these reactions in seawater are well known (Millero et al., 2002; Fabry et al., 2008).

Elevated pCO₂ of seawater (i.e. hypercapnia) could cause deleterious effects on marine organisms through reduced calcium carbonate (CaCO₃) saturation, resulting in low calcification rates, and disturbance to acid-base (metabolic) physiology. Current studies also reveal the uptake of anthropogenic CO₂ by the ocean, and the resultant alterations in the base (metabolic) physiology. Current studies also reveal the uptake of

Altered pCO₂ levels within the range of predicted near-future ocean acidification scenarios of ~700–1,500 μatm pCO₂ increases heavy metal solubility and metal (Ni, Zn, and Fe) influx from the sediments into the water column (Breitbarth et al., 2010; Roberts et al., 2013). Also, mobilization of Cd, Cu, Pb and Zn into the water column increases at lowered pH of 7.5 and 6.5 depending on the binding strength between the metal and the sediment particles with the degree of mobilization dependent on the strength of the association (Riba et al., 2003).

The desorption of heavy metals from sediments into water column could be enhanced by the ocean acidification-induced reduction in biological calcification and dissolution of CaCO₃ thus leading to the local increase in the concentrations of Ca²⁺ in the water, and also resulting in an enhanced release of heavy metal pollutants by competing with Ca²⁺ and H⁺ for their binding sites (Du Laing et al., 2009). Metal speciation strongly influences heavy metal bioavailability due to the free ionic forms usually being highly bioavailable (Paquin et al., 2000). Speciation of numerous metals that form strong complexes with OH and CO₃⁻ ions are predicted to be significantly affected by ocean acidification (Millero et al., 2009). Concentrations of OH⁻ and CO₃⁻ reduce with ocean acidification resulting in higher concentrations of the most bioavailable free ionic form of these metals (Ivanina and Sokolova, 2015). Copper and nickel are examples of metals that form strong complexes with CO₃⁻; therefore, ocean acidification-induced reduction in CO₃⁻ levels will invariably cause an increase in free Cu²⁺ and Ni²⁺ concentrations (from the present-day ~ 8 % and 4 % to ~32 % and 13 % by the year 2250 for Cu²⁺ and Ni²⁺, respectively) (Millero et al., 2009). Free iron concentrations (Fe²⁺) are expected to increase from the current 66 %–90 % by the year 2250, while the speciation of other heavy metals is projected to be less affected by ocean acidification (Ivanina and Sokolova, 2015). The proportion of free Pb²⁺ is expected to increase from ~3 % to ~6 % by the year 2250 (pH 7.4) (Millero et al., 2009), while speciation of the metals which predominantly form complexes with chloride (e.g. Cd²⁺ and Hg²⁺) are insensitive to ocean acidification (Millero et al., 2009).

Recent studies on the effects of seawater pCO₂/pH on uptake and accumulation of heavy metals, an intricate pattern which cannot be easily predicted from the chemical models of metal speciation and ligand binding is depicted (Ivanina and Sokolova, 2015). The effects of pCO₂/pH on uptake, accumulation and toxicity of metals are dependent on the species, organism’s life stage and the OA levels rather than the predicted concentrations of the free metals in seawater (Ivanina and Sokolova, 2015). Uptake and accumulation of Cd, one of the most studied metals about ocean acidification-metal interactions could serve as a useful reference illustration for species and environment-dependent variability of responses to pCO₂. Cadmium speciation, unlike Cu and Fe, is independent of pH and pCO₂ (Ivanina and Sokolova, 2015).

The physiological effects of OA in many marine organisms have been extensively studied (Das and Mangwani, 2015), but the potential for OA to interact with other environmental stressors remains poorly understood (Crain et al., 2008). Till date, such studies have focused on combining OA with either temperature, salinity or hypoxia (Lewis et al., 2016). Of particular interest for environmental assessment, however, is the understanding of how near-future OA will change the behaviour and bioavailability of persistent marine contaminants, notably heavy metals. Studies by Lewis et al. (2016), revealed that near-future OA scenarios significantly increases the sub-lethal toxicity responses of two key coastal marine invertebrates, namely mussels (Mytilus edulis) andurchins (Paracentrotus lividus) to relevant concentrations of copper in the marine environment. Copper-induced damage to DNA of both marine invertebrates was significantly greater when the animals were exposed to nominal 0.1 μm copper under OA (high pCO₂/low pH) conditions compared with animals exposed under extant pCO₂ levels (Lewis et al., 2016).

2. Materials and methods

2.1. Crab sampling

Dotilla fenestra (N = 540; 7 ± 1 mm carapace width) collected from Durban Bay Harbour, KwaZulu-Natal in line with the recommended ethical and governmental requirements, were cleaned with filtered seawater to remove debris and washed with 30 % artificial seawater to eliminate unwanted contaminants. They were subsequently acclimated in a constant temperature room at 18 °C and 32 psu, controlled photoperiodic duration of 12L:12D with a pH of 8.1 for 72 h.

2.2. Experimental setup

The acute exposures of crabs were carried out according to the standard methodology such as those of the FAQ (Ward and Parrish, 1982; Reitsch and Oshida, 1987) and the American Public Health Association (Apha, 1992). Prior to exposures and preparations of stock solution, all glassware was soaked in 10 % nitric acid and rinsed thoroughly with
double distilled water and deionized water. A three by three experimental grid design was adopted, and a range-finder test was run to select applicable metal concentrations for the crabs to be exposed to in 96 h. Crabs were exposed to three varying metal concentrations and pH in triplicates per combined concentrations of metal and pH by bubbling CO₂ using an automated CO₂ system into glass tanks containing 5 L of (Fatoki and Mathabatha, 2001) homogenized stock solutions of sub-lethal concentrations of Cd (0.5, 0.75 and 1.0 mg/l), Pb (6.5, 8.5 and 10.5 mg/l) and Cd & Pb (4.5 (0.50 Cd & 4.00 Pb), 5.75 (0.75 Cd & 5.00 Pb) and 7.0 (1.00 Cd & 6.00 Pb) mg/l) freshly prepared by dissolving the appropriate analytical grade of metal salts CdCl₂.₅H₂O for Cd, and Pb(NO₃)₂ for Pb in deionized water with standard glass flasks. These sub-lethal concentrations fall between the range of Cd and Pb concentrations obtained by Fatoki and Mathabatha (2001) in water and sediment from East London, and Port Elizabeth Harbours in South Africa and represent about 1000 % order of magnitude above the South African marine water quality guideline target values (Cd 4.0 μg/g and Pb 12.0 μg/g). Stock solutions were acidified by the controlled bubbling of CO₂ into stock solution (Chapman, 1978) to obtain varying pH groups of 7.2, 7.4 and 7.6 to simulate predicted near-future coastal pH. Tanks were covered with plastic lids to minimize evaporation and small holes bored through the centre to allow for bubbling of air and CO₂ throughout the experiment. Test solutions were set up and running 24 h before the introduction of crabs. The experiment was conducted in a constant temperature room at 18 °C and 32 psu and a controlled photoperiodic duration of 12L:12D. Before each experiment, 20 active crabs were gently introduced into each tank. Water quality parameters were monitored periodically throughout the 96-hour duration of the experiment to ensure that all variables were within experimental limits. At the end of the experiment, haemolymph was extracted for osmolality determination, and the remaining crabs were stored in a freezer for metal analysis.

2.3. Sample preparation and analysis

Crabs were thawed and dissected for the tissue – exoskeleton (as the tissue compartment with the most significant metal accumulation (Adeleke, 2017)). Dissected tissue was weighed, and oven-dried at 50 °C to constant weight for at least 48 h. About 0.5g of homogenized dried tissues were crushed to uniform particle size with a lab porcelain mortar and pestle and digested in 20 ml concentrated AR grade nitric acid for at least 24 h. Subsequently, the digested samples were mixed with 10 ml of concentrated AR grade nitric and perchloric acid (4:1), heated on a hot plate at 120 °C and subsequently made up to 20 ml by adding 20 ml solution of Milli-Q water with 20 % nitric acid and filtered with Whatman filter paper (Sudharsan et al., 2012). Heavy metal concentrations were determined using ICP-OES, Perkin Elmer optima 5300 DV and crab tissues were analyzed for Cd and Pb using crab paste (LGC 7164) as certified reference material to test for analytical accuracy of the metals as represented in Table 1.

2.4. Statistical analysis

Main effects analysis of variance (ANOVA) and pairwise comparisons analysis using Tukey's HSD test were used to test for a statistical difference in mean metal concentrations in crab tissue due to combined effects of pH and metal. Pearson's correlation coefficient was used to determine the relationship between mean metal concentrations in the crab tissue and varying pH using Statistica 13.0 software program.

3. Results

Mean cadmium concentrations in the exoskeleton of crabs exposed to a different combination of acute exposures of Cd and pH media occurred in the order of 7.4 > 7.6 > 7.2 (Figure 1 and Table 2). Cadmium concentration in the exoskeleton of crabs exposed to pH 7.4 with Cd 0.50 mg/l concentration was significantly higher (ANOVA HSD: df 6; p < 0.01) compared with those exposed to pH 7.2 and 7.6. There was, however, no significant difference (ANOVA HSD: df 6; p > 0.05) between the Cd levels in the exoskeleton of crabs in media pH 7.2 and 7.6 (Table 2).

Bioconcentration of Pb in the crabs differed with increasing concentrations of Pb in the water medium and showed different trends with varying pH (Figure 2). Concentration of Pb in the exoskeleton of crabs exposed to concentrations of 10.50 mg/l Pb with pH 7.6 was significantly higher (ANOVA HSD: df 6; p < 0.01) compared to those at pH 7.4/10.50 mg/l, while those exposed to pH 7.2/10.50 mg/l were significantly higher (ANOVA HSD; df 6; p < 0.01) compared to those exposed to pH 7.4/10.50 mg/l. Crabs exposed to Pb concentrations of 6.50 mg/l and 8.50 mg/l showed no significant variation (ANOVA HSD; df 6; p > 0.05) in the exoskeleton's levels of Pb with varying pH (see Figure 2 and Table 2).

Bioconcentration of Cd and Pb in the exoskeleton of crabs exposed to mixed metal Cd/Pb differed with varying pH compared to those exposed to either Cd or Pb only (Figures 1, 2, and 3). Cadmium and Pb concentrations in the exoskeleton of crabs exposed to media concentrations of 4.50 mg/l and 7.0 mg/l of mixed Cd/Pb with pH 7.2 were significantly higher (ANOVA HSD: df 6; p < 0.01) compared with those at pH 7.4 and 7.6, while Pb concentration in the exoskeleton of crabs exposed to 5.75 mg/l Cd/Pb with pH 7.4 was significantly higher (ANOVA HSD: df 6; p < 0.01) than those at pH 7.2/5.75 mg/l and pH 7.6/5.75 mg/l.

Figure 1. Mean Cd concentrations (μg/g ± SD) in the exoskeleton of D. fenestrata after 96 h acute exposure to 0.50, 0.75 and 1.00 mg/l Cd solutions.
Cd/Pb concentrations. There was no significant difference (ANOVA HSD: df 6; p > 0.05) in Cd concentration in the exoskeleton of crabs exposed to Cd/Pb concentration of 5.75 mg/l with different pH (Figure 3 and Table 2).

The Pearson's Correlation coefficient between the exoskeleton of the crabs exposed to combinations of varying pH of 7.2, 7.4 and 7.6 and concentrations of heavy metals (Cd, Pb and Cd/Pb) did not show any significant correlation (p > 0.05) in this study (Table 3).
Ocean/coastal acidification is a developing concern in marine ecosystems due to rising atmospheric CO2 concentrations and projected to affect speciation of heavy metals considerably thus resulting to the high bioavailability of the free ionic metals (Ivanina and Sokolova, 2015). Crustaceans accumulate trace metals in their tissues with high variability across different metals (Rainbow, 2007). Current studies on the effects of OA on uptake and concentrations of heavy metals reveal a complex form that is difficult to predict from metal speciation and ligand binding chemical models hence, the effects of PCO2/OA on bioaccumulation of metals and toxicity are mainly reliant on species, organism life stage and level of acidification rather than the levels of predicted bioavailability of metals in seawater (Ivanina and Sokolova, 2015). This study shows that at pH 7.4, mobilization and concentrations of Cd in the exoskeleton of D. fenestrata increases significantly compared to pH of 7.2 and 7.6. Concentrations of Cd in the exoskeleton of the crabs were not directly proportional to elevated CO2 concentration in the water column (Figure 1). These results corroborate those of (Millero et al., 2009) Millero et al. (2009) which showed metals such as Cu+2, Cd+2, and Hg+2 that form strong complexes with chloride experience little or no alteration in speciation as a result of chloride concentration not being affected by lowering pH. Cadmium bioconcentrations in marine organisms represent a good reference for species and environmentally dependent hypercapnia variability, as Cd is a widely studied metal with regards to metal-OA interactions (Ivanina and Sokolova, 2015). Cadmium speciation is not dependent on pH and PCO2 compared to Cu and Fe, hence the bioavailability of free Cd ions are not the major factor influencing Cd uptake by organisms, instead of the interactions of complex factors which are largely dependent on species and levels of OA (Ivanina and Sokolova, 2015) (see Table 4). Uptake of Cd from the water column into mantle tissues of marine bivalves Mercenaria mercenaria and Crassostrea virginica increased with increasing levels of CO2 climaxing at intermediate PCO2 (~800 μatm) in oysters and extreme PCO2 (~2,000 μatm) in clams (Götze et al., 2014). The observed changes probably showed the effects of PCO2 on physiological uptake and distribution of Cd due to the suppression of Cd accumulation in clams as a result of high PCO2 isolated in their mantle (Ivanina et al., 2013). Uptake of Cd from sediment in clam Ruditapes philippinarum and an amphipod Corophium volutator juveniles were independent of PCO2 (López et al., 2010; Roberts et al., 2013). Also, elevated PCO2 reduced Cd uptake by tissue compartments of sea anemone Anemonia viridis (Horwitz et al., 2014). Therefore, a comprehensive biochemical model that can describe the effects of OA on metal bioavailability and toxicity to aquatic organisms is still doubtful due to metal diversity and hypercapnia interactions combined with the lack of a strong correlation between the PCO2-dependent metal speciation and uptake (Ivanina and Sokolova, 2015).

4. Discussion

Ocean/coastal acidification is a developing concern in marine ecosystems due to rising atmospheric CO2 concentrations and projected to affect speciation of heavy metals considerably thus resulting to the high bioavailability of the free ionic metals (Ivanina and Sokolova, 2015). Crustaceans accumulate trace metals in their tissues with high variability across different metals (Rainbow, 2007). Current studies on the effects of OA on uptake and concentrations of heavy metals reveal a complex form that is difficult to predict from metal speciation and ligand binding chemical models hence, the effects of PCO2/OA on bioaccumulation of metals and toxicity are mainly reliant on species, organism life stage and level of acidification rather than the levels of predicted bioavailability of metals in seawater (Ivanina and Sokolova, 2015). This study shows that at pH 7.4, mobilization and concentrations of Cd in the exoskeleton of D. fenestrata increases significantly compared to pH of 7.2 and 7.6. Concentrations of Cd in the exoskeleton of the crabs were not directly proportional to elevated CO2 concentration in the water column (Figure 1). These results corroborate those of (Millero et al., 2009) Millero et al. (2009) which showed metals such as Cu+2, Cd+2, and Hg+2 that form strong complexes with chloride experience little or no alteration in speciation as a result of chloride concentration not being affected by lowering pH. Cadmium bioconcentrations in marine organisms represent a good reference for species and environmentally dependent hypercapnia variability, as Cd is a widely studied metal with regards to metal-OA interactions (Ivanina and Sokolova, 2015). Cadmium speciation is not dependent on pH and PCO2 compared to Cu and Fe, hence the bioavailability of free Cd ions are not the major factor influencing Cd uptake by organisms, instead of the interactions of complex factors which are largely dependent on species and levels of OA (Ivanina and Sokolova, 2015) (see Table 4). Uptake of Cd from the water column into mantle tissues of marine bivalves Mercenaria mercenaria and Crassostrea virginica increased with increasing levels of CO2 climaxing at intermediate PCO2 (~800 μatm) in oysters and extreme PCO2 (~2,000 μatm) in clams (Götze et al., 2014). The observed changes probably showed the effects of PCO2 on physiological uptake and distribution of Cd due to the suppression of Cd accumulation in clams as a result of high PCO2 isolated in their mantle (Ivanina et al., 2013). Uptake of Cd from sediment in clam Ruditapes philippinarum and an amphipod Corophium volutator juveniles were independent of PCO2 (López et al., 2010; Roberts et al., 2013). Also, elevated PCO2 reduced Cd uptake by tissue compartments of sea anemone Anemonia viridis (Horwitz et al., 2014). Therefore, a comprehensive biochemical model that can describe the effects of OA on metal bioavailability and toxicity to aquatic organisms is still doubtful due to metal diversity and hypercapnia interactions combined with the lack of a strong correlation between the PCO2-dependent metal speciation and uptake (Ivanina and Sokolova, 2015).
Lead concentration in the crab’s exoskeleton in this study did not follow a defined order as accumulation in the exoskeleton varies with levels of Pb exposure and pH. While accumulation in crabs exposed to 6.50 mg/l Pb concentrations demonstrated increased Pb accumulation with increasing pH, those exposed to 8.50 mg/l accumulated more Pb with decreasing pH and those exposed to 10.50 mg/l exhibited an intermediate uptake and distribution behaviour to those exposed to 6.50 and 8.50 mg/l Pb concentrations. This could be due to the effects of the transitional behaviour of Pb, which enables it to form significant complexes with both Cl⁻ and CO₃²⁻ (see Table 4). Therefore, as pH decreases, the free ion form of Pb increases by ~10% resulting in a great increase in its complexation with Cl⁻ (15% among PbCl₂, PbCl₃, and PbCl₄) (Millero et al., 2009). The physiology of the crab as it relates to absorption capacity, metabolism and modification of regulatory capacity when exposed to Pb only (Núñez-Nogueira et al., 2012) might also play a role.

Many studies on the accumulation of Pb in marine organisms have broadly shown that uptake and bioaccumulation of Pb are mainly dependent on the level of bioavailability (Chinni et al., 2000). However, elevated concentration of Pb was observed in the exoskeleton of the crabs exposed to a metal mixture of Cd and Pb (Figure 3). This observation corroborates the findings of Núñez-Nogueira et al. (2012), who observed a significant elevation in body concentrations of Pb in shrimp Peneaus vannamei exposed to the mixed treatment of Pb and other metals. Elevated body concentration was detected when different metals competed at the same time, seemingly due to synergic effects stimulated by the occurrence of other metals in the water column. It can be inferred from the results that exposure of crabs to metal mixture aids metal uptake from the water column in several orders of magnitude, and body regulatory capacity seems to be compromised with combined metal uptake. Several studies have also demonstrated metal regulatory capacities of many marine crustaceans when exposed to mixed metal concentrations; Panulirus inflatus (Paez-Osuna et al., 1995), Macrobrachium malcolmsonii and Penaeus indicus (Vijayram and Geraldine, 1996) and Litopenaeus vannamei exhibited regulation to specific metal exposure levels of 0.2 mg/l (Wu and Chen, 2005). As observed from this study, crabs exposed to mixed metal (Cd/Pb) concentrations mostly have significantly elevated concentrations of Cd and Pb at pH of 7.2 compared to those at pH 7.4 and 7.6 except for crabs exposed to 5.75 mg/l water concentrations of Cd/Pb.

5. Conclusion

Ocean acidification affects the dynamics of heavy metals in seawater and will lower the pH of estuarine waters, thus altering the biogeochemical processes in these systems resulting in greater changes in metal speciation. The solubility of various metals in seawater is mainly dependent on pH and is affected by changes in speciation, which result in modifications to behaviour and release the fate of metals in the marine environment. The combined effects of these factors are mostly responsible for bioavailability, uptake, accumulation and toxicity of metals to aquatic organisms. This study shows that Cd uptake and accumulation in crab tissues were significantly elevated at pH of 7.4 and not directly proportional with decreasing pH, indicating that Cd mobilization in the water column and subsequent uptake by crabs was significantly higher at pH 7.4 compared to pH 7.2 and 7.6. Lead uptake is dependent on bioavailability levels and the presence of other competing metals while accumulation in crab tissues is also dependent on regulatory physiology of the organism.

Declarations

Author contribution statement

Babatunde Ayoade Adeleke: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Deborah Robertson-Andersson, Gan Moody: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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