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INITIAL EVALUATION OF METAL CONTENT OF *Acanthaster planci* AND *Linckia laevigata* COLLECTED FROM CARMEN, AGUSAN DEL NORTE, PHILIPPINES

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Abstract

Harmful substances like metals ultimately end in the seafloor and contaminate marine ecosystems. These substances are of particular concern due to their bioavailability and toxicity to marine organisms. In this study, metals (Pb, Cd, Cr, Zn, and Cu) were determined in the body wall of common sea star *L. laevigata*, and *A. planci* collected from Carmen, Agusan del Norte, Philippines. The metal content in terms of concentration of *L. laevigata* follows the order Pb > Cu > Cd > Cr > Zn while for *A. planci* is Pb > Cu > Cr > Zn > Cd. Both species were found to consistently contain the most lead and copper, which could suggest bioaccumulation of these elements from the environment. It is believed that these metals were sourced from the sediments or their food and are accumulated in their body wall. The differences in metal content for Pb and Cd were believed to be due to the difference in the diet for the two species. Comparable metal content was found in Cd, Cr, and Zn. It is recommended that further studies are conducted, especially in monitoring the metal content in the sediment and bioaccumulation of these metals in different trophic levels.

INTRODUCTION

The seafloor is considered as the final acceptor for many contaminants (Matranga et al., 2012). These contaminants are often carried by rivers or the atmosphere and eventually reach and contaminate marine environments (Danis et al., 2006). An example of these contaminants is heavy metals. Heavy metal ions are insoluble substances dissolved in water, which forms toxic sediments at the bottom of bodies of water (Walag, Canencia, et al., 2018). These toxic substances often bring undesirable effects to organisms at different levels of the food chain and by disrupting the functions of other essential metals (Jakimska et al., 2011a). Monitoring of heavy metals in the environment is important in maintaining healthy river systems including waterbodies that are near mining industries, electroplating, tanning, and other similar industries (Baharom & Ishak, 2015; Canencia et al., 2016; Canencia & Walag, 2016).

Marine organisms are also used to monitor heavy metal contamination. This is due to their varied coping mechanisms to respond to environmental stresses like heavy metal contamination. Echinoderms are accepted worldwide as excellent ecotoxicological and genotoxicological models since they are directly exposed to emerging anthropogenic contaminants for both in their plankton and benthic stage in their life cycles (Falugi et al., 2012). Moreover, echinoderms are valuable indicators of heavy metal contamination as they accumulate it as a function of the level of contamination in the environment (Coteur et al., 2003). Although echinoderms are considered to be bioaccumulators of heavy metals, the process by which heavy metals are accumulated in organisms is considered to be species-dependent thus, we may find a variety of organism having different levels of heavy metals (Jakimska et al., 2011a).Sea stars are considered to be good detectors of contamination due to their position in the food chain serving as either primary consumer or secondary consumer. In one study, bioaccumulation of Cd and Pb is dependent on the levels of concentrations of these metals in the environment *A. rubens* (Temara, Warnau, Jangoux, et al., 1997). This sea star is capable of translocating heavy metals to different compartments of the body and that the rate of bioaccumulation depends on the body compartment (Temara, Warnau, Duboisa, et al., 1997).

Understanding the heavy metal concentration in the sea star could help people understand the degree of contamination of marine ecosystems since they are known to be bioaccumulators of heavy metals. In the Philippines, no study has been conducted yet specifically on the heavy metal content of *L. laevigata* and *A. planci* and even other sea star species. Existing current studies are on their ecology (Llacuna et al., 2016; Walag, Layaog, et al., 2018; Walag & Canencia, 2016), biochemical composition (Walag & Del Rosario, 2018), antimicrobial and cytotoxicity (Layson et al., 2014), metabolite screening (Walag et al., 2019), and antioxidant activity (Walag & Del Rosario, 2020). In the Environmental Management Bureau (EMB) report, 63 bodies of water have been monitored for metal contamination in Mindanao Island of the Philippines (Walag, Canencia, et al., 2018). It is for this reason that this study aimed to determine the metal content of *L. laevigata* and *A. planci*, collected from Carmen, Agusan del Norte, in terms of lead, cadmium, chromium, zinc, and copper.

MATERIALS AND METHODS

Study Area

The animal materials required for this study was collected from the intertidal zone up to shallow waters (0-5m) of Barangay Goso-on, Carmen, Agusan del Norte, Philippines. The map of the collection site is shown in Figure 1. The Municipality of Carmen is located in the province of Agusan del Norte of Caraga Region or Region XIII. It is strategically located along the Western Agusan Corregidor, surrounded by the Butuan Bay in the north, Buenavista in the south, Nasipit in the east, and Misamis Oriental in the west.

Several mining industries were also noted to be present in Agusan del Norte. Their respective locations and distance from the sampling area is shown in Figure 12. According to the unpublished report from the Mines and Geosciences Bureau of the Department of Environment and Natural Resources in 2017, there are 5 mining industries with Mineral Production Sharing Agreement (MPSA) while the other 5 mining industries are with Exploration Permit (EP) in the province of Agusan del Norte. Out of the 5 mining industries, there are 2 that are considered to be in active operation in Agusan del Norte. These are SR Metals Inc. and Agata Mining Ventures Inc. shown in Figure 2. SR Metals Inc. is known to operate on nickel, cobalt, iron, and other associated mineral deposits and has an expiration date of March 10, 2033 for commercial operation while December 23, 2034 for exploration on nickel and other associated mineral deposits. Agata Mining Ventures Inc. operates on copper and gold and possesses and MPSA with an expiration date of May 26, 2024, approving a development plan covering a 600-hectare portion of land. SR Metals Inc is 91 kilometers away from the sampling area while Agata Mining Ventures Inc. is 112 kilometers away.

Figure 1. Geographic location of the collection site in Barangay Gosoon, Carmen (C), with respect to the Philippines (A) and Agusan del Norte (B).

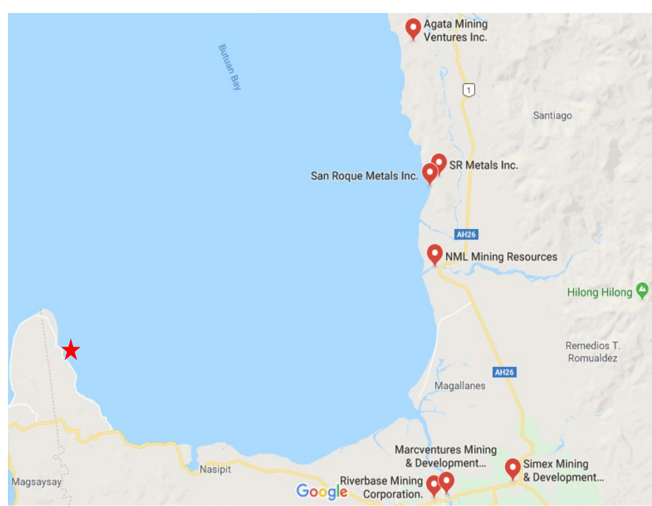

Figure 2. Mining industries in Agusan del Norte surrounding the Butuan Bay relative to the sampling site.

Identification and Preparation of Sea Star Specimens

Field reconnaissance was conducted to evaluate the presence and abundance of marine sea stars selected for this study. The procedures for the collection and identification of specimens were based on the work of Walag et al. (2019). Gratuitous Permit (GP-2019-01) was also acquired from the Bureau of Fisheries and Aquatic Resources – Caraga Region before sampling and reconnaissance.

Fresh samples were collected during the lowest low tide of April 2019 from the intertidal zone up to shallow (3-5meters deep) parts of the marine environment in Barangays Goso-on and Vinapor, Carmen, Agusan del Norte. Mature (>5inches across) sea stars that were collected were washed and then placed in a styrobox with ice and marine water for preservation. All collected samples were brought to Cagayan de Oro City for storage and further sample processing.

Heavy Metal Content Determination

The heavy metals present in the samples were analyzed using AAS spectrophotometric method (Besagas et al., 2015). Briefly, the solid samples were subjected to dry ashing in the furnace at 600ºC, and about 0.5 grams were extracted from microwave digestion (Sineo Microwave Digestion). This was digested with 8.0 mL of the concentrated HNO_3 for pre-treatment in 20 minutes. It was further digested with concentrated HNO_3 and 30% H_2O_2. It was then filtered when already cooled using a Whatman no. 42 filter paper and was diluted to 100 mL with deionized water. The concentrations of lead, cadmium, and chromium were determined using a PerkinElmer AAnalyst 200 atomic absorption spectrophotometer with acetylene gas as fuel.

Statistical Analyses

All tests were performed in triplicates. Ten individuals for both species served as the crude sample. The data on the concentration of heavy metals were analyzed using an independent t-test. All quantitative data were presented as mean ± standard deviation, and the differences were considered at 0.05 level of significance.

RESULTS AND DISCUSSION

Lead

The presence of heavy metals in the body of sea stars is not unusual nor surprising since echinoderms have been accepted globally as indicators since they accumulate metals as a function of the level of contamination in the environment (Coteur et al., 2003). Higher levels of Pb present in the sea star samples was found in this study compared to the study on *A. rubens* (Coteur et al., 2003; Danis et al., 2004, 2006), where < 2ppm of total lead for both pyloric caeca and body wall were found in various sampling regions. In addition, high levels of lead in sea stars are not surprising since, in a certain study, Pb was found to bioconcentrate most on *A. rubens* (Fedyunin et al., 2019). Although sea stars are known to be bioaccumulators of heavy metals, the process in which heavy metals are accumulated in organisms is considered to be species-dependent since they possess relatively different biochemical processes (Jitar et al., 2015).

This could also signify that higher levels of lead can be found in the environment where the sea stars were collected compared to the sampling area of the different literatures mentioned. Butuan Bay is considered to be the final destination of various water bodies in Agusan del Norte, where several active and inactive mining sites are found. Moreover, various studies have also determined high levels of lead in the different water bodies that feed the Butuan Bay (Cabuga et al., 2017).

A higher concentration of Pb was noted in the *A. planci* compared to *L. Laevigata*, as shown in Table 1. Differences in Pb concentration in the two species is expected since the process by which heavy metals are accumulated in organisms is considered to be species-dependent thus we may find a variety of organism having different levels of heavy metals (Jakimska et al., 2011a). Moreover, the difference in heavy metal content could also be due to the difference in the diet of the two sea stars. *A. planci* is known to be a prolific corallivore, which means that they feed mainly on corals. At the same time, *L. laevigata* is an opportunistic predator and scavenger, feeding on marine worms, algae, and other detritus. This could be the reason why *A. planci* is recorded to have higher Pb content since some species of corals are accumulators of Pb (Tanaka et al., 2013). Lead in sea stars has been efficiently

Table 1. The metal content of two sea star species collected from Carmen, Agusan del Norte

Species	Metals (ppm)				
	Pb	Cd	Cr	Zn	Cu
L. laevigata	24.00 ± 5.49	17.08 ± 0.54	13.23 ± 2.74	4.09 ± 2.98	21.44 ± 0.46
A. planci	40.38 ± 5.90	4.53 ± 0.14	9.46 ± 1.99	6.79 ± 1.17	21.95 ± 0.56
p-value	0.024*	0.000*	0.126^{NS}	0.218^{NS}	0.286^{NS}

*Significant at 0.05 level, ^{NS}Not significant incorporated in calcitic skeletons (Temara et al., 1998). This could be one of the reasons for the differences in the Pb content of the two sea stars. As reported, *A. planci* has more ash content (mineral content) compared to *L. laevigata*, thus could be the reason for the higher Pb content of *A. planci* than *L. laevigata* (Walag & Del Rosario, 2018).

Cadmium

The presence of Cd is expected since literature noted the presence of this metal in various parts of the sea star *A. rubens* (Coteur et al., 2003; Danis et al., 2004, 2006). This means that sea stars are capable of accumulating cadmium in the different parts of their body. Similar results were also noted in the literature mentioned that Cd was the second highest in terms of dry weight concentration. The Cd concentration also seemed to be higher compared to *A. rubens* pyloric caeca, ranging from 1.0 to 3.0 ppm (den Besten et al., 2001) especially to *L. laevigata* even if the values will be multiplied to a factor of 2.5 (since the whole body was considered in this study and not pyloric caeca alone) as suggested in the literature (den Besten et al., 1991) but comparable to *A. planci*. Although the difference in Cd content may be associated with the sea star's ability to accumulate metals, literature suggested that this could be simply due to seasonal variation which influences gametogenesis and accumulation of metals and lipophilic contaminants (den Besten et al., 2001). The gametogenesis of sea stars often starts in August/September and peaking in April (den Besten et al., 2001). During this stage, metals and lipophilic contaminants tend to increase in the pyloric caeca as part of this reproductive cycle, as suggested in the literature (den Besten et al., 1990). The sea star samples of this study were collected in April 2019, which could be the reason for the high levels of Cd.

A higher concentration of Cd was noted in *L. Laevigata* compared to *A. planci*, as shown in Table 1. Differences in Cd concentration in the two species is expected since the process by which heavy metals are accumulated in organisms is considered to be species-dependent thus we may find a variety of organism having different levels of heavy metals (Jakimska et al., 2011a, 2011b). Moreover, the difference in heavy metal content could also be attributed to the difference in the diet of the two sea stars. As mentioned earlier, *L. laevigata* feeds on algae which are known to bioaccumulate Cd (Kola & Wilkinson, 2005; Maeda et al., 1990) while *A. planci* feeds on corals which slowly absorbs Cd (Chan et al., 2012).

Chromium

The presence of Cr in sea stars is difficult to corroborate due to the very limited literature detailing how it is accumulated and sourced by them. However, several pieces of literature exist on other marine invertebrates and sea stars from polar regions. The concentration of Cr for the two sea stars ranges from 7.157 to 15.70 ppm, which is comparable to Cr in various invertebrates collected from Admiralty Bay (Trevizani et al., 2016) and Terra Nova Bay (Grotti et al., 2008). The results of this study do not conform to the Cr content of *N. armata*, which ranges from 1.4 to 2.7 ppm (Duquesne & Riddle, 2002). The difference could be due to the fact that *N. armata* thrives in Polar Regions while the sea stars collected in this present study thrives in tropical to temperate regions. The presence of Cr in these species is also expected since a study (Cabuga et al., 2017) noted the accumulation of Cr in a flathead grey mullet from a nearby body of water where the sampling was done. Moreover, echinoderms were also noted to accumulate Cr least compared to arthropods and mollusks and that this low accumulation of Cr may not pose a threat to the organism itself but rather towards its predator (Ju et al., 2016).

There is no significant difference noted in the Cr content for both sea stars. This could be due to the fact that one of the reasons for differences in metal content is based on the animal's diet. Both corals and algae have been found to accumulate Cr (Anu et al., 2007; Dwivedi et al., 2010; Gorbi et al., 2001; Ranjbar Jafarabadi et al., 2018), thus both sea star species may have sourced Cr from their diet.

Zinc

The presence of zinc in sea stars is not surprising since literature have reported high zinc content in the sea star *A. rubens* (Brügmann & Lange, 1988; Danis et al., 2006). This result indicates that both sea stars are capable of accumulating Zn in their body parts. However, compared to the mentioned studies, the zinc content in this present study is relatively lower. The low content of zinc is also surprising since this metal is considered as an essential element that accumulates fast (Brügmann & Lange, 1988). This could also signify that the Zn content in the sampling area may not be that high since metal accumulation is a function of the level of contamination in the environment (Coteur et al., 2003). Low levels of zinc were also noted in the coastal areas of Misamis Oriental (Besagas et al., 2015), which is a neighboring province of the sampling area. The low level of zinc compared to other studies could be due to the observed large sizes of the samples. As suggested, zinc content has a significant negative correlation with dry weight and maximum diameter (Brügmann & Lange, 1988). Samples of both species collected for this study had a diameter greater than 5 inches. Zn content for both species has been noted to be comparable. This is expected since Zn is essential in animal diet which possesses important metabolic and neural functions.

Copper

Copper content found in this present study is relatively higher compared to the literature on *A. rubens* (Brügmann & Lange, 1988; Danis et al., 2004). This result is expected since high Cu content was found in sediments of Agusan River, which empties on Butuan Bay (Cabuga Jr et al., 2016). Also, higher Cu content was noted in the arms of *A. rubens* compared to its central disc with a ratio of 1.30 (Brügmann & Lange, 1988). This could explain the high content of Cu in this study since most of the material used for this study is study is the body wall. This could further suggest that Cu is mostly deposited in as part of the protein complex and calcitic skeletons in the body walls similar to Pb, as suggested by Temara et al. (1998). In addition, the high concentration of Cu is not surprising since, in a certain study, Cu was found to bioconcentrate highly in *A. rubens* (Fedyunin et al., 2019). This implies that Cu accumulation is not unusual to sea stars and may further suggest that this element could have an important biochemical function in the organism. Moreover, Cu is also known to be an essential trace mineral in animals which possesses important roles in collagen formation, iron absorption, and in energy production.

CONCLUSION

The metal content of *L. laevigata* and *A. planci* collected from Carmen, Agusan del Norte, Philippines, was determined using the standard AAS spectrophotometric method. Metals considered were Pb, Cd, Cr, Zn, and Cu. Both species had the highest in terms of Pb and Cu content while lowest in Zn for *L. laevigata* while Cd for *A. planci*. The presence and difference of these metals in the samples were believed to be due to their diet, sediment metal concentration, and ability to link the metal in protein complexes and calcitic skeletons. The high concentration of Pb and Cu could mean possible bioaccumulation of these metals from the environment, although this was not determined in the study. Based on these findings, it is recommended that further studies be done in unraveling the biochemical process of accumulation of metals in these species to deeply understand their biology as indicator species.

REFERENCES

Anu, G., Kumar, N. C., Jayalakshmi, K. J., & Nair, S. M. (2007). Monitoring of heavy metal partitioning in reef corals of Lakshadweep Archipelago, Indian Ocean. *Environmental Monitoring and Assessment*. https://doi.org/10.1007/s10661-006-9305-7

Baharom, Z. S., & Ishak, M. Y. (2015). Determination of Heavy Metal Accumulation in Fish Species in Galas River, Kelantan and Beranang Mining Pool, Selangor. *Procedia Environmental Sciences*, *30*, 320–325. https://doi.org/10.1016/j.proenv.2015.10.057

Besagas, R. L., Asoy, A. Y., Ceniza, M. S., Leopoldo, G. D., Dael, N. T., & Del Rosario, R. M. (2015). Upland and Coastal Freshwater Sources in Misamis Oriental, Philippines. *Mindanao Journal of Science and Technology*, *13*.

Brügmann, L., & Lange, D. (1988). Trace metal studies on the starfish Asterias rubens L. from the western Baltic Sea. *Chemistry and Ecology*, *3*(4), 295–311.

Cabuga, C. J. C., Seronay, R. A., Busia, M. A., Billuga, N. P., Ayaton, M. A., Angco, M. K. A., Joseph, C. C. D., Guilleno, E. R., Colon, R. M. A., Havana, H. C., Budlayan, M. L. M., & Arriza, S. M. (2017). Geometric morphometric and heavy metals analysis of flathead grey mullet (Mugil cephalus), from Agusan River, Butuan city, Philippines. *Journal of Biodiversity and Environmental Sciences*, *11*(1), 134–151.

Cabuga Jr, C. C., Velasco, J. P. B., Leones, J. A. M., Orog, B. Y., & Jumawan, J. C. (2016). Levels of cadmium, copper, lead, nickel and mercury in the muscles of Pigok (Mesopristes cancellatus) and sediments collected at lower Agusan river basin, Brgy. Pagatpatan, Butuan City, Agusan Del Norte, Philippines. *International Journal of Fisheries and Aquatic Studies*, *4*(4), 206–215.

Canencia, O. P., Dalugdug, M. D., Emano, A. M. B., Mendoza, R. C., & Walag, A. M. P. (2016). Slaughter waste effluents and river catchment watershed contamination in Cagayan de Oro City , Philippines. *Journal of Biodiversity and Environmental Sciences*, *9*(2), 142–148.

Canencia, O. P., & Walag, A. M. P. (2016). Coal combustion from power plant industry in Misamis Oriental, Philippines: A potential groundwater contamination and heavy metal detection. *Asian Journal of Microbiology, Biotechnology, and Environmental Sciences*, *18*(1), 55–59. http://www.envirobiotechjournals.com/article_abstract.php?aid=6730&iid=208&jid=1

Chan, I., Tseng, L. C., Kâ, S., Chang, C. F., & Hwang, J. S. (2012). An experimental study of the response of the gorgonian coral Subergorgia suberosa to polluted seawater from a former coastal mining site in Taiwan. *Zoological Studies*, *51*(1), 27–37.

Coteur, G., Gosselin, P., Wantier, P., Chambost-Manciet, Y., Danis, B., Pernet, P., Warnau, M., & Dubois, P. (2003). Echinoderms as bioindicators, bioassays, and impact assessment tools of sediment-associated metals and PCBs in the North Sea. *Archives of Environmental Contamination and Toxicology*, *45*(2), 190–202. https://doi.org/10.1007/s00244-003-0199-x

Danis, B., Wantier, P., Dutrieux, S., Flammang, R., Dubois, P., & Warnau, M. (2004). Contaminant levels in sediments and asteroids (Asterias rubens L., Echinodermata) from the Belgian coast and Scheldt estuary: Polychlorinated biphenyls and heavy metals. *Science of the Total Environment*. https://doi.org/10.1016/j.scitotenv.2004.05.002

Danis, B., Wantier, P., Flammang, R., Pernet, P., Chambost-Manciet, Y., Coteur, G., Warnau, M., & Dubois, P. (2006). Bioaccumulation and effects of PCBs and heavy metals in sea stars (Asterias rubens, L.) from the North Sea: A small scale perspective. *Science of the Total Environment*, *356*(1–3), 275–289. https://doi.org/10.1016/j.scitotenv.2005.05.029

den Besten, P. ., Valk, S., van Weerlee, E., Nolting, R. ., Postma, J. ., & Everaarts, J. . (2001). Bioaccumulation and biomarkers in the sea star Asterias rubens (Echinodermata: Asteroidea): a North Sea field study. *Marine Environmental Research*, *51*(4), 365–387. https://doi.org/10.1016/S0141-1136(00)00134-3

den Besten, P. J., Bosma, P. T., Herwig, H. J., Zandee, D. I., & Voogt, P. A. (1991). Effects of cadmium on metal composition and adenylate energy charge in the sea starAsterias rubens L. *Archives of Environmental*

Contamination and Toxicology, *21*(1), 112–117. https://doi.org/10.1007/BF01055565

den Besten, P. J., Herwig, H. J., Zandee, D. I., & Voogt, P. A. (1990). Cadmium Accumulation and Metallothionein-like Proteins in the Sea Star Asterias Asterias rubens. *Arch. Environ. Contam. Toxicol*, *19*(9960), 858–862.

Duquesne, S., & Riddle, M. J. (2002). Biological monitoring of heavy-metal contamination in coastal waters off Casey Station, Windmill Islands, East Antarctica. *Polar Biology*, *25*(3), 206–215. https://doi.org/https://doi.org/10.1007/s00300-001-0328-9

Dwivedi, S., Srivastava, S., Mishra, S., Kumar, A., Tripathi, R. D., Rai, U. N., Dave, R., Tripathi, P., Charkrabarty, D., & Trivedi, P. K. (2010). Characterization of native microalgal strains for their chromium bioaccumulation potential: Phytoplankton response in polluted habitats. *Journal of Hazardous Materials*, *173*(1–3), 95–101. https://doi.org/10.1016/j.jhazmat.2009.08.053

Falugi, C., Aluigi, M. G., Chiantore, M. C., Privitera, D., Ramoino, P., Gatti, M. A., Fabrizi, A., Pinsino, A., & Matranga, V. (2012). Toxicity of metal oxide nanoparticles in immune cells of the sea urchin. *Marine Environmental Research*, *76*(114–121). https://doi.org/10.1016/j.marenvres.2011.10.003

Fedyunin, V. A., Poromov, A. A., & Smurov, A. V. (2019). The Effect of Metal Ions on Survival and Cellular Elements of Coelomic Fluid of Starfish Asterias rubens (Linnaeus, 1758) (Echinodermata: Asteroidea). *Contemporary Problems of Ecology*, *12*(4), 386–393. https://doi.org/10.1134/S1995425519040048

Gorbi, G., Corradi, M. G., Invidia, M., & Bassi, M. (2001). Light Intensity Influences Chromium Bioaccumulation and Toxicity in Scenedesmus acutus (Chlorophyceae). *Ecotoxicology and Environmental Safety*, *48*(1), 36–42. https://doi.org/10.1006/eesa.2000.2000

Grotti, M., Soggia, F., Lagomarsino, C., Riva, S. D., Goessler, W., & Francesconi, K. A. (2008). Natural variability and distribution of trace elements in marine organisms from Antarctic coastal environments. *Antarctic Science*. https://doi.org/10.1017/S0954102007000831

Jakimska, A., Konieczka, P., Skóra, K., & Namieśnik, J. (2011a). Bioaccumulation of metals in tissues of marine animals, part I: the role and impact of heavy metals on organism. *Polish Journal of Environmental Studies*, *20*(5), 1117–1125.

Jakimska, A., Konieczka, P., Skóra, K., & Namieśnik, J. (2011b). Bioaccumulation of metals in tissues of marine animals, part II: Metal concentrations in animal tissues. *Polish Journal of Environmental Studies*, *20*(5), 1127–1146.

Jitar, O., Teodosiu, C., Oros, A., Plavan, G., & Nicoara, M. (2015). Bioaccumulation of heavy metals in marine organisms from the Romanian sector of the Black Sea. *New Biotechnology*, *32*(3), 369–378. https://doi.org/10.1016/j.nbt.2014.11.004

Ju, Y.-R., Chen, C.-W., Chen, C.-F., & Dong, C.-D. (2016). Metal accumulation in benthic invertebrates and sediments at the Kaohsiung Ocean Disposal Site, Taiwan. *Desalination and Water Treatment*, *57*(60), 29254–29263. https://doi.org/10.1080/19443994.2016.1138148

Kola, H., & Wilkinson, K. J. (2005). Cadmium Uptake by a Green Alga Can Be Predicted by Equilibrium Modelling. *Environmental Science & Technology*, *39*(9), 3040–3047. https://doi.org/10.1021/es048655d

Layson, R. J., Rodil, M. C. A., Mojica, E. E., & Deocaris, C. C. (2014). Potential Anti-cancer and Anti-bacterial Activities of Philippine Echinoderm Extracts. *J. Trop. Life. Science*, *4*(3), 175–181.

Llacuna, M. E. J., Walag, A. M. P., & Villaluz, E. (2016). Diversity and dispersion patterns of echinoderms in Babanlagan, Talisayan, Misamis Oriental, Philippines. *Environmental and Experimental Biology*, *14*(4), 213–217. https://doi.org/10.22364/eeb.14.28

Maeda, S., Mizoguchi, M., Ohki, A., & Takeshita, T. (1990). Bioaccumulation of zinc and cadmium in freshwater alga, . Part I. Toxicity and accumulation. *Chemosphere*, *21*(8), 953–963. https://doi.org/10.1016/0045-6535(90)90118-D

Matranga, V., Pinsino, A., Randazzo, D., Giallongo, A., & Dubois, P. (2012). Long-term environmental exposure to metals (Cu, Cd, Pb, Zn) activates the immune cell stress response in the common European sea star (Asterias rubens). *Marine Environmental Research*, *76*, 122–127. https://doi.org/10.1016/j.marenvres.2011.09.008

Ranjbar Jafarabadi, A., Riyahi Bakhtiari, A., Maisano, M., Pereira, P., & Cappello, T. (2018). First record of bioaccumulation and bioconcentration of metals in Scleractinian corals and their algal symbionts from Kharg and Lark coral reefs (Persian Gulf, Iran). *Science of the Total Environment*. https://doi.org/10.1016/j.scitotenv.2018.06.029

Tanaka, K., Ohde, S., Cohen, M. D., Snidvongs, A., Ganmanee, M., & McLeod, C. W. (2013). Metal contents of Porites corals from Khang Khao Island, Gulf of Thailand: Anthropogenic input of river runoff into a coral reef from urbanized areas, Bangkok. *Applied Geochemistry*. https://doi.org/10.1016/j.apgeochem.2013.07.005

Temara, A., Skei, J. M., Gillan, D., Warnau, M., Jangoux, M., & Dubois, P. (1998). Validation of the asteroid Asterias rubens (Echinodermata) as a bioindicator of spatial and temporal trends of Pb, Cd, and Zn contamination in the field. *Marine Environmental Research*. https://doi.org/10.1016/S0141-1136(98)00026-9

Temara, A., Warnau, M., Duboisa, P., & Langston, W. J. (1997). Quantification of metallothioneins in the common asteroid Asterias rubens (Echinodermata) exposed experimentally or naturally to cadmium. *Aquatic Toxicology*, *38*(1–3), 17–34. https://doi.org/10.1016/S0166-445X(96)00844-2

Temara, A., Warnau, M., Jangoux, M., & Dubois, P. (1997). Factors influencing the concentrations of heavy metals in the asteroid Asterias rubens L. (Echinodermata). *Science of the Total Environment*, *203*(1), 51–63. https://doi.org/10.1016/S0048-9697(97)00134-4

Trevizani, T. H., Figueira, R. C. L., Ribeiro, A. P., Theophilo, C. Y. S., Majer, A. P., Petti, M. A. V., Corbisier, T. N., & Montone, R. C. (2016). Bioaccumulation of heavy metals in marine organisms and sediments from Admiralty Bay, King George Island, Antarctica. *Marine Pollution Bulletin*. https://doi.org/10.1016/j.marpolbul.2016.02.056

Walag, A. M. P., & Canencia, M. O. P. (2016). Physico-chemical parameters and macrobenthic invertebrates of the intertidal zone of Gusa, Cagayan de Oro City, Philippines. *AES Bioflux*, *8*(1), 71–82. http://www.aes.bioflux.com.ro/docs/2016.71-82.pdf

Walag, A. M. P., Canencia, O. P., & Fiedler, B. A. (2018). Water Quality: Mindanao Island of the Philippines. In B. A. Fiedler (Ed.), *Translating National Policy to Improve Environmental Conditions Impacting Public Health Through Community Planning* (pp. 219–253). Springer International Publishing. https://doi.org/10.1007/978-3-319-75361-4_12

Walag, A. M. P., & Del Rosario, R. M. (2018). Proximate biochemical composition and brine shrimp lethality assay of selected sea stars from Goso-on and Vinapor, Carmen , Agusan del Norte, Philippines. *Malaysian Journal of Biochemistry and Molecular Biology*, *21*(3), 11–18.

Walag, A. M. P., & Del Rosario, R. M. (2020). Total Flavonoids Content, Total Phenolics Content, and Antioxidant Activities of Acanthaster planci and Linckia laevigata collected from Carmen, Agusan del Norte, Philippines. *Malaysian Journal of Biochemistry and Molecular Biology*, *23*(1), 77–85.

Walag, A. M. P., Del Rosario, R. M., & Canencia, O. P. (2019). Zoochemical Composition of Selected Sea Stars Collected from the Coastal Waters of Carmen, Agusan Del Norte, Philippines. *Asian Journal of Biological and Life Sciences*, *8*(2), 53–62. https://doi.org/10.5530/ajbls.2019.8.10

Walag, A. M. P., Layaog, A. G., & Garcia, G. U. (2018). Survey of echinoderms in the intertidal zone of Goso-on and Vinapor, Carmen, Agusan del Norte, Philippines. *Environmental and Experimental Biology*, *16*(1), 31–38. https://doi.org/10.22364/eeb.16.04