# BCR SEQUENTIAL EXTRACTION FOR GEOCHEMICAL FRACTIONS AND ASSESSMENT OF ZN, NI, CD, CU, AND PB IN THE COASTAL SEDIMENTS, IN THE SANTOS PORT COMPLEX, SOUTHEAST BRAZIL

**Jéssica de F. Delgado**
jessiicafdelgado@gmail.com
Fluminense Federal University - UFF, Niteroi, RJ, Brazil.

**Leonardo da S. Lima**
leodslima@gmail.com
Fluminense Federal University - UFF, Niteroi, RJ, Brazil.

**Khauê Vieira**
kvieira87@gmail.com
Fluminense Federal University - UFF, Niteroi, RJ, Brazil.

**José Antônio Baptista Neto**
jabneto@id.uff.br
Fluminense Federal University - UFF, Niteroi, RJ, Brazil.

**Estefan Monteiro da Fonseca**
oceano25@hotmail.com
Fluminense Federal University - UFF, Niteroi, RJ, Brazil.

## ABSTRACT

Water column variances in the aquatic environment, motivated by either natural or anthropogenic processes, may impact the stability of trace metals in the sediment of Santos Port Complex (SPC), located in southeast Brazil. Trace metals such as Cu, Zn, Ni, Pb, and Cd potentially negatively affect ecological and biological compartments in estuarine water. To evaluate the status of the sediment of the SPC, the present article investigated the geochemical fraction of sedimentary trace metals by applying the BCR (European Community Bureau of Reference) sequential extraction approach. Organic matter and fine grain size, characteristic of an estuarine environment, showed a direct influence on the concentration dynamics of trace metals in sediments, especially for Pb, Cd, and Ni. In the specific case of Zn and Cu, there was a potential source of contamination in the port. The largest fraction of the metals studied was non-residual, potentially mobilizable in an environment where variation in the physicochemical parameters of the water column is highly variable, something frequent in estuarine environments.

**Keywords:** trace metal speciation; Santos Harbor; sediment; environmental impact.

## INTRODUCTION

As a result of the nature of port activities, transport complexes are generally installed in geomorphologically protected areas, safe from strong open sea hydrodynamics. In addition, for the beneficiation, transformation, and circulation of transported raw materials and industrial products that arrive and leave the portuary site constantly, an industrial park is installed nearby, resulting in the consequent human settlement of the entire region. Thus, natural confined sites such as estuaries and bays are suitable environments for developing this type of activity (Iglesias *et al*., 2021). On the other hand, estuaries and bays, due to their strong water physic-chemical gradients derived from the transition between freshwater on the continent and salty marine waters, present a great biodiversity, representing one of the most productive ecosystems around the planet (Barbier *et al*., 2011; Pinto and Marques, 2015). Additionally, the calm water body works as a nursery for marine organisms that use this environment to reproduce and feed (Kotzé, 2016). Therefore, despite their great ecological importance and thus a high degree of sensitivity, port regions represent one of the environments most altered by human activities (Gillanders *et al*., 2011; Vermeiren and Sheaves, 2014; Kotzé, 2016). Among the most critical impacting factors existing in the day-to-day life of the ports are the constant dredging, waste disposal, constant transit of vessels, high population density, and waste generation, among others.

The natural characteristics of estuarine environments make these ecosystems even more complex from an ecological point of view (Sun *et al*., 2015; Zhang M. *et al*., 2017; Duggan *et al*., 2019; Izegaegbe *et al*., 2020). Their geomorphologically protected water, with low hydrodynamic fluxes and restricted water exchange with the adjacent sea, results in lower oxygen levels in the water column, which is propitious to the concentration of contaminants on its sediment, composed of fine-grained particles and high levels of organic matter (Guerra-García and García-Góme 2004). So, many ports are known as critically polluted sites, representing a real threat to the local living organisms (Galkus *et al*., 2012; Zaaboub *et al*., 2014).

The marine environment has become polluted due to a wide range of contaminants sourced from several anthropogenic activities, and this has prompted worldwide attention for a long time (Adekunle, 2012; Eliku and Leta, 2018). Trace metal pollution in estuarine ecosystems is an increasing challenge, as most trace metals beyond threshold concentrations are potentially toxic to living organisms (Prabhakaran *et al*., 2016). These elements can find their own ways to enter the food web in the environment. However, the toxicity and associated damage of individual metals are relatively well studied with established baselines (Yi *et al*., 2021).

Estuarine ecosystems are highly dynamic sites that are often coupled with seawater dilution effects and various anthropogenic inputs. All environmental parameters can then further affect trace metal bioassimilation since they influence interactions between heavy metals and the aquatic biota. Thus, the comprehension of trace metal bioavailability is not an easy task; it requires the integration of all major contributing factors, such as different environmental variables, multi-heavy metal mixtures, and their speciation (Gu and Gao, 2021), when attempting to investigate processes that dictate the relation between trace metals and the biota.

Total levels of trace metal recording in the sedimentary pool do not represent an effective indicator when the object is the evaluation between natural and anthropogenic sources (Relic *et al*., 2010, Passos *et al*., 2011, Okoro *et al*., 2012) and its potential bioavailability (Zhong *et al*., 2011). For this purpose, recent authors have applied sequential partial extraction approaches to evaluate the specific chemical forms associated with sedimentary phases and metal sources (Hang *et al*., 2009; Davutluoglu *et al*., 2011).

One commonly used sequential extraction approach is the BCR (European Community Bureau of Reference) method. The BCR method divides trace metals into four groups: exchangeable and easily soluble fractions, easily reducible fractions, oxidizable fractions, and residual fractions. The first three fractions theoretically represent the potentially available fractions that under physicochemical fluctuations of the water column, are made available. The last represents a difficult fraction to mobilize (Sahara *et al*., 2015), considered naturally inert.

The main object of the present study was to diagnose the status of sedimentary trace metals to allow the dynamic projection of the geochemical distribution under variations of the physicochemical parameters of the water column in the Santos Estuarine System (SES).

### Study site

The SES (Figure 1) consists of a coastal region severely modified by countless human activities of the most diverse nature, resulting in several potential pollution sources, including a vast industrial complex, intensely populated areas, and agricultural, and portuary activities. One of the most significant sources is Cubatão industrial park, located in the Santos watershed basin (Azevedo Netto *et al*., 2022), which concentrates petrochemical, steel, chemicals, fertilizers, and logistics, as well as energy production and services and whose activities present potential punctual and diffuse sources of pollutants. Parallelly, the same region is under the influence of irregular depositions of industrial solid waste caused by port activities, sewage treatment stations,

submarine outfalls, and clandestine discharges of domestic sewage and sanitary landfills. Finally, SUS is still the target of constant impacts generated by the maintenance of the Port of Santos, the largest port complex in Latin America, where periodical dredging activities are carried out (Figure 1). On the other hand, the coastal region receives many tourists during the summer (Azevedo Netto *et al*., 2022), with traditional fishing being an important economic activity in the region (Hortellani *et al*., 2008).

## METHODOLOGY

### Sampling

A sampling campaign was carried out on May 21. 32 sampling spots were distributed along the main estuarine channel, as shown in Figure 1. Water column physicochemical characterization was made at surface and bottom water depths during the ebb tide. Salinity was evaluated with a multiparameter Horiba U10 probe.

Additionally, with the same approach as Netto *et al*. (2021), past data was also used to reinforce the accuracy of the physical analysis of the waters. The average of all data groups used was compared with the trace metal concentrations and other environmental parameters (pH, Eh, dissolved oxygen, and salinity).

A stainless Van Veen grab was used to maintain sediment composition integrity and avoid contamination during sediment sample collection. Then the samples were properly packaged and stored under refrigeration (~-20 ºC) until analysis. The following analysis was carried out on the sediment samples: the sediment grain size, total organic carbon (TOC) levels, phosphorus concentrations, and metals (Ni, Cu, Zn, Cr, and Pb) were determined.

### Laboratorial Analysis

Trace metal samples were maintained in pre-acidified plastic bowls and transported to the laboratory for analysis. For the grain size evaluation, a pre-treatment process was used, degrading the organic matter with hydrogen peroxide ($H_2O_2$). Posteriorly, the grain size was measured by a Microtrac S3500 grain size analyzer. The results were classified into sand, silt, and clay according to the sediment composition.

Then, the fine material (below 0.063 mm) was used to extract the trace metals. Sequential extraction was performed using the BCR approach. After samples were centrifuged and further diluted, trace metals (Pb, Cu, Ni, Zn, and Cd) were analyzed by Inductively Coupled Plasma-Atomic Emission Spectrometry. The detection limits of the method are, respectively: Pb, 15 mg/kg; Cu, 1.5 mg.kg-1; Ni, 0.1 mg.kg-1; Zn, 0.4 mg.kg-1; and Cd, 0.4 mg.kg-1.
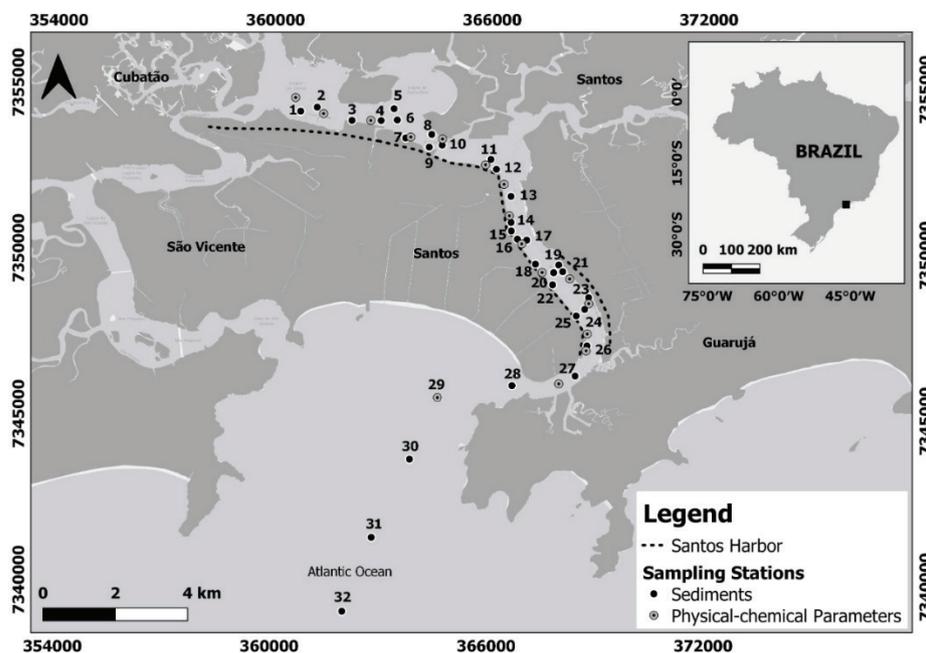


**Figure 1.** Study area and sampling stations

## Statistical analysis

The normal distribution of the data was tested with the Shapiro-Wilk test. Spearman's correlation was used to evaluate the relationship between trace metal data and environmental parameters.

## RESULTS AND DISCUSSION

Sediment trace metal levels represent potentially reasonable indicators of the state of an ecosystem (Forstner and Wittmann, 1981). Once trace metals enter the estuary, they promptly become associated with the particulate phase and sink to the subaquatic bottom (Hanson *et al.*, 1983). The decantation process depends on several water column features, such as pH, Eh, ionic strength, organic and inorganic matrixes, and the available surface area for adsorption caused by variation in grain size distribution (Davis *et al.*, 1991). The combination of trace metals with fine particles (silt and clay) is attributed to co-precipitation or complexation reactions of these elements on particle surfaces, determining the distribution pattern of trace metals in sedimentary pools (Ho *et al.*, 2010). In the present study, grain size records are presented in Figure 2. Size is a fundamental property of sediment particles. It determines particle diffusion and deposition through the environment and thus provides relevant information about the sediment's provenance, transport history, and depositional conditions (Kenneth Pye, 2004). In the present study, the finer grain size fraction was present along all the sampling stations, being more concentrated in the estuarine inward area, where the lower hydrodynamic conditions permitted its deposition.

Sediments consist of multi-phase matrixes composed of silicates, carbonates, hydroxides/oxides, sulfates, and organic substances as major components (Zhu, 2006). Organic matter, in turn, shows the capacity to retain, reduce mobility, and consequently reduce trace metal bioavailability (Im-

pellitteri *et al.*, 2002; Wolińska *et al.*, 2018). Therefore, organic compounds play an important role in forming complexes and retaining heavy metals in a potentially mobilizable form (Aydinalp and Marinova, 2003).

In the present study, the organic matter levels varied between 3,5 and 22,5%. Contrary to expectations, the higher concentrations were in Santos's channel due to the greater currents in this area. On the other hand, the water salinity, proper from open sea water, may promote estuarine flocculation and aggregation mechanisms, carrying the dissolved organic matter to the subaquatic bottom pool (Boyle *et al.*, 1977; Sholkovitz, 1978; Davis, 1984; Gibbs, 1986). Thus, the estuarine physico-chemical particle settlement mechanism, together with the existence of a submarine outfall and the potentially active processes of flocculation, can explain this distribution.

Among the pollutants present in estuarine environments, a critical role is played by trace elements, which, under certain water column conditions, are potentially toxic to living species and are transmitted through the trophic chain (Rosado *et al.*, 2016a, b; Tarnawski and Baran, 2018). Trace elements deposited in bottom sediments can potentially threat aquatic organisms. Moreover, in the case of improper sediment management, e.g., from the dredging of port evolution basin sediment, they can also pose the danger of having a toxic impact on land biota.

Some authors demonstrated that flocculation of dissolved organic matter in estuarine environments may enhance sedimentation of organic-metal complexes derived from terrestrial ecosystems (Sholkovitz, 1978; Sholkovitz *et al.*, 1978; Wells *et al.*, 2000; Turner *et al.*, 2002; Stolpe and Hassellöv, 2007; Biati *et al.*, 2010; Samani *et al.*, 2014; Heidari, 2019). As seen in Figure 3, total trace metal concentration data revealed two distribution patterns. The first one was followed by Ni, Pb, and Cd, which showed the same appearance as organic matter levels. This data reveals the importance of
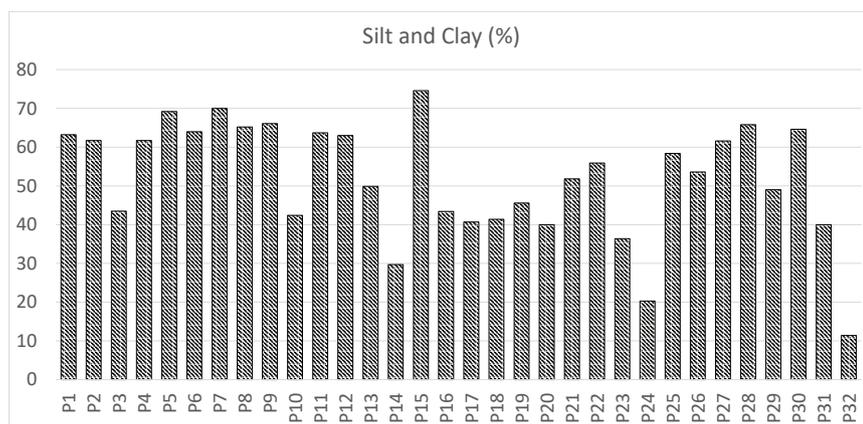


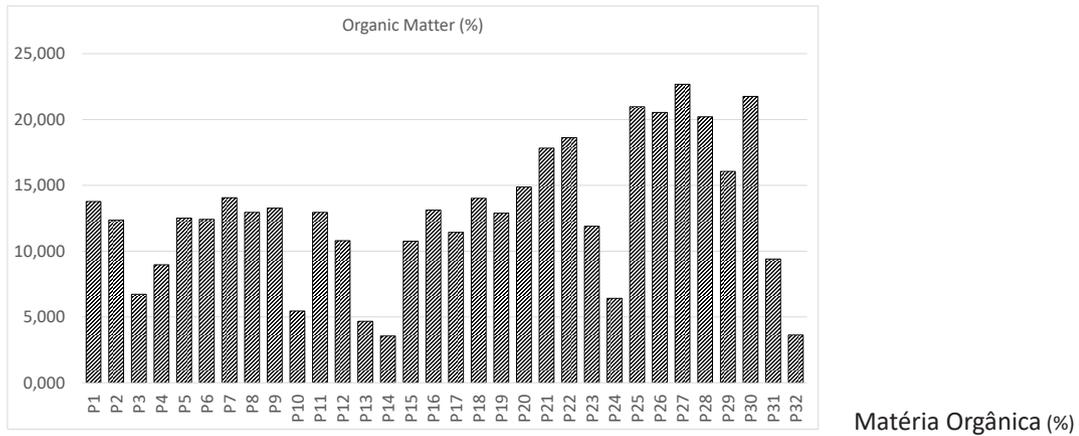**Figure 2.** Fine-grained particle distribution

**Figure 3.** Organic Matter distribution

the organic matter in the heavy metal accumulation in the Santos Estuarine System.

The second pattern, followed by Zn and Cu, did not follow the deposition pattern promoted by organic matter affinity and concentration. The similarity between these trace metals and their random character suggested port activity as a potential source for these trace metals. Copper mining and processing are fundamental for infrastructure development. As a result, highly industrialized areas use the present Cu contamination from metallurgical waste (Wang *et al.*, 2015). More important than that, copper and zinc are used in antifouling paints as active ingredients and stabilizers, respectively (Bighiu *et al.*, 2017).
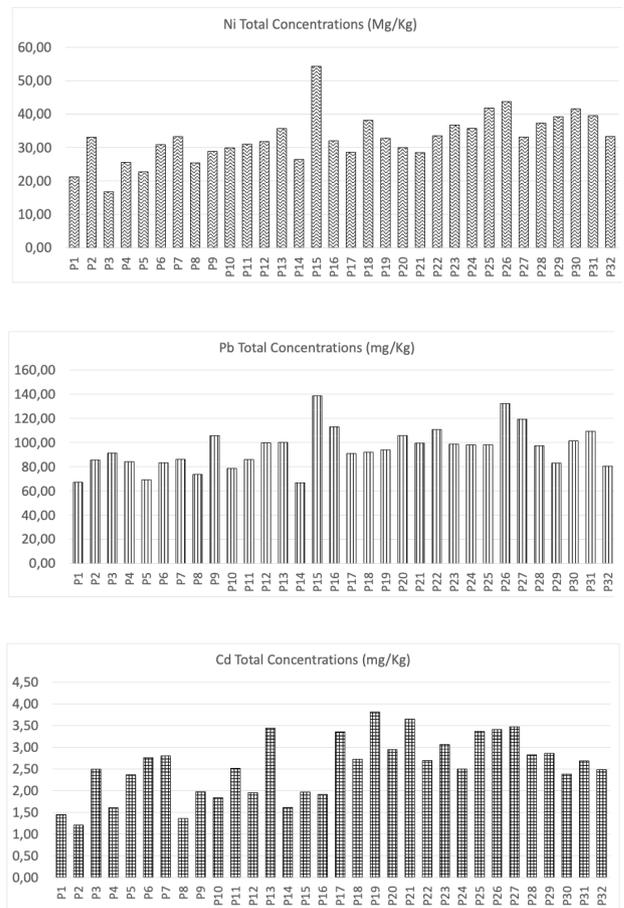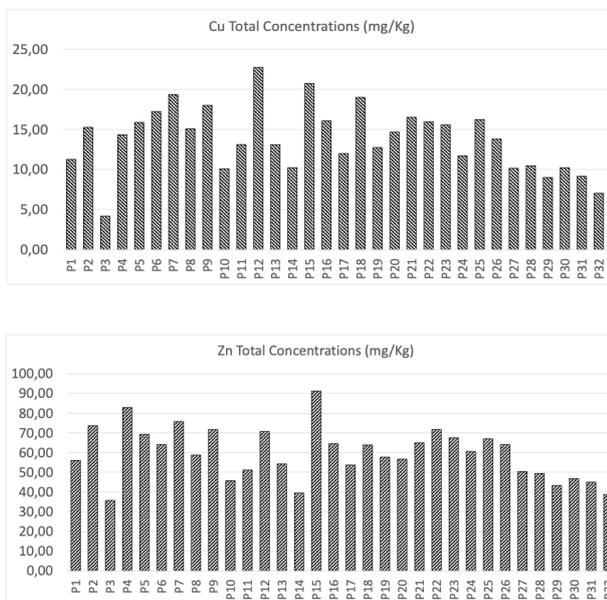




**Figure 3.** Total trace metal concentrations

Table 1 presents the statistical analysis results. According to the obtained data, hydrodynamic fluxes represent the main distribution parameter for local floating particles since fine grain size and organic matter have a significant correlation. The same can be said for Cu and Zn since both metals presented significant correlations with fine-grained particles. On the other hand, organic matter showed to be preponderant for Cd and Pb sediment accumulation.

**Table 1.** Spearman test results

| | Fine Grain size | Mo (%) | Cu (Total) | Zn (Total) | Ni (Total) | Pb (Total) | Cd (Total) |
|---|---|---|---|---|---|---|---|
| Fine fraction | | 0.392 | 0.453 | 0.470 | -0.062 | 0.004 | -0.213 |
| Mo (%) 0.392 | | | 0.217 | 0.160 | 0.288 | 0.361 | 0.399 |
| Cu (Total) | 0.453 | 0.217 | | 0.868 | 0.051 | 0.262 | -0.020 |
| Zn (Total) | 0.470 | 0.160 | 0.868 | | 0.051 | 0.257 | -0.117 |
| Ni (Total) | -0.062 | 0.288 | 0.051 | 0.051 | | 0.546 | 0.384 |
| Pb (Total) | 0.004 | 0.361 | 0.262 | 0.257 | 0.546 | | 0.407 |
| Cd (Total) | -0.213 | 0.399 | -0.020 | -0.117 | 0.384 | 0.407 | |

Sedimentary trace metal fractionation reflects the metal binding processes in sediments. The dynamic of metal fractionation in these sediments is a result of the differences between each metal source and its particular affinity with environmental matrixes and particular physicochemical responses. Generally, metals sourced from human activities are presented in the potentially mobilizable fractions, whereas metals derived from natural geologic sources are in the residual fractions (Passos *et al.*, 2010).
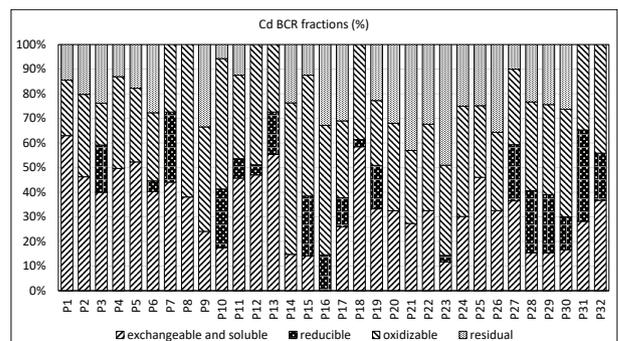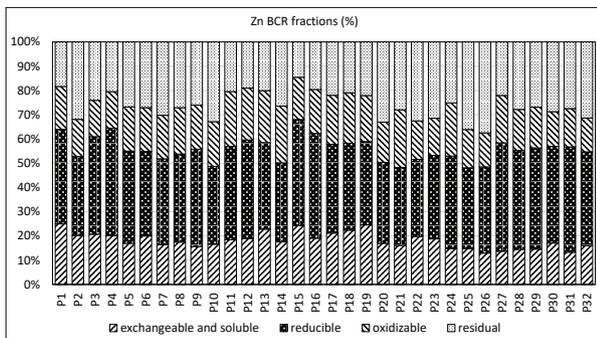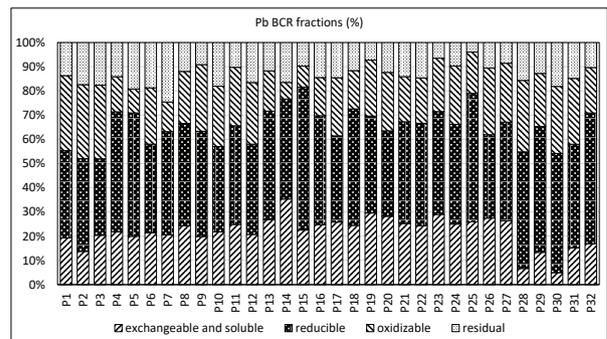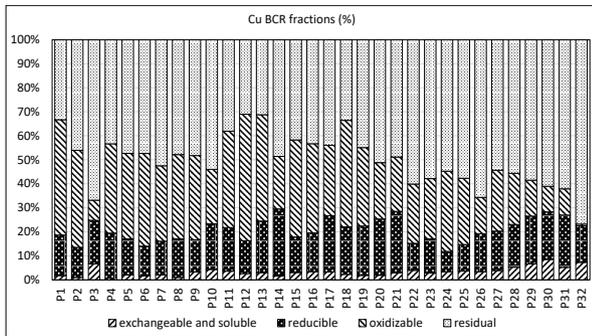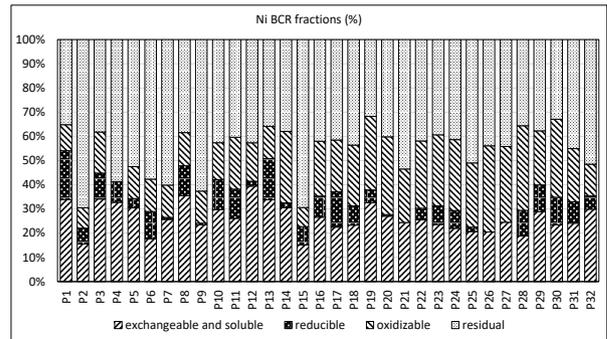


Figure 4. Studied trace metal BCR fractions

In the present study, a significant amount of all studied metals was associated with the non-residual phase, indicating that all trace metals are potentially bioavailable. In the non-residual sediment fraction, the speciation status and potential bioavailability of trace metals primarily depend on the binding of these elements to solid particles, their presence in ionic form in the water column, or their being adsorbed on the surfaces of sediment mineral/organic matter/microorganisms complexes. In this sense, the results showed that the subaquatic bottom's stored trace metals may be remobilized from the sedimentary pool via natural or anthropogenic mechanisms. The first hypothesis may be the result of the water column's physicochemical conditions variation (e.g., pH, redox potential, organic matter, and salinity) (Wu *et al*., 2015), as a result of estuarine tidal and saline wedge oscillations. On the other hand, the constant dredging work necessary to maintain the depths in the vessels' evolution basin can affect the stability of the bottom sediments and thus remobilize the pollutants deposited back into the water column (Monte *et al*., 2019). So, according to the present study, especially Cd and Pb showed less association with the most stable fraction.

In aquatic bodies, the non-residual trace metals are transported as dissolved, colloidal, or particulate elements or compounds associated with available natural matrixes like organic matter, Fe and Mn (oxyhydr) oxides, and clay particles, which are omnipresent in aquatic ecosystems and present a high metal sorption ability (Singh and Subramanian, 1984; Parker and Rae, 1998; Warren and Haack, 2001; Pokrovsky and Schott, 2002; Hassellöv and von der Kammer, 2008; Nystrand *et al*., 2012). According to the present study, the organic phase recorded omnipresence for all the studied trace metals as a result of the organic matter's significant availability in estuarine areas, where the high productivity and the water exchange restriction permit its accumulation. After organic matter enters the environment, it produces trace metal organic complexes due to abundant carboxyl, hydroxyl, amine, and other organic particle surface reactive groups, increasing its adsorption capacity (Kulikowska *et al*., 2015; Zhou *et al*., 2015). Some authors have suggested that Cu is able to form complexes with organic matter (Manceau and Matynia, 2010; Moon and Peacock, 2012; Karlsson *et al*., 2006) or organic matter-copper mineral ternary complexes (Strawn and Baker, 2009). So, in the present study, sequential extraction analysis confirmed organic matter as an important matrix immobilizing local Cu. Recorded data in the present article suggested that the dynamic of the other studied trace metal mobility is also associated with high organic matter concentrations, as shown in Figure 4.

In the same way that Cu, Pb, and Zn have great affinity with the hydroxyl functional group available on mineral surfaces like kaolinite, oxides, oxyhydroxides, and Fe and Al hydroxides (Smith *et al*., 1995). According to Silveira *et al*. (2003), iron oxides are the most relevant soil constituents for retaining heavy metals. The authors' affirmation is applicable to the present case, except for Ni and Cd, trace metals that recorded negligible participation in the reducible phase in most sampling stations. According to Qion *et al*. (2020) and Ma *et al*. (2021), sediment minerals are the main constituents of solid sediment matrices, and clay minerals are the most impacting elements in sediments that primarily include layered silicate minerals and crystalline and amorphous oxide matrixes that significantly influence the immobility and diffusion of trace metals.

The most dangerous fraction of trace metals stays in the free ionic species, which are highly mobile and easily bioavailable (Sunda and Lewis, 1978; Luoma, 1983). In the present study, this fraction was shown to be less important for Cu. The other metals, on the other hand, recorded a significant contribution from the soluble or exchangeable fraction.

## CONCLUSIONS

Heavy metals are increasingly present in the environment, representing risks to ecological health when in anomalous concentrations. Their total concentrations do not allow for the projection of the potentially deleterious effects these elements offer. Its toxicity is associated with the particular characteristics of each metal and the ligand matrices available in the environment since these will define the mobility of trace metals. In the present study, metals were present at unnatural levels in most sampling stations. Organic matter and fine grain size, characteristic of an estuarine environment, directly influenced the concentration dynamics of trace metals in sediments, especially for Pb, Cd, and Ni. In the specific case of Zn and Cu, there was a potential source of contamination in the port. The largest fraction of the metals studied was non-residual, potentially mobilizable in an environment where variation in the physicochemical parameters of the water column is highly variable, something frequent in estuarine environments. More studies are needed to assess the effective negative effects of metals on the Santos estuarine ecosystem.

## REFERENCES

Adekunle, A.S., Odukoya, O.O., Ayenimo, J.G., Oyekunle, J.A.O., Doherty, W.O., Mamba B.B. & Akanni, M.S. (2012), "Removal of heavy metals from industrial effluents by water hyacinth (Eichornia crassipes)", *Journal of Environmental Chemistry and Ecotoxicology*, vol. 4, no. 11, pp. 203-2011, DOI: 10.5897/JECE12.037

Aydinalp, C. & Marinova, S. (2003), "Distribution and forms of heavy metals in some agricultural soils", *Polish Journal of Environmental Studies*, vol. 12, no. 5, pp. 629–633.

Azevedo Netto, A., Souza, P.F., Lima, L.S., Vieira, K.S., Delgado, J.F, Menezes, C.R., Neves, C.V, Baptista Neto, J.A. & Fonseca, E.M. (2022), "Dinâmica de distribuição e fontes potenciais de hidrocarbonetos aromáticos policíclicos para sedimentos de superfície e bivalves de um estuário altamente antropizado", *Sistemas & Gestão*, vol. 17, no. 1, pp. 104-117, DOI: 10.20985/1980-5160.2022.v17n1.1790

Barbier, E.B., Hacker, S.D., Kennedy, C., Koch, E.W., Stier, A.C., & Silliman, B.R. (2011), "The Value of Estuarine and Coastal Ecosystem Services", *Ecological Monographs*, vol. 81, no. 2, pp. 169–193, DOI: 10.1890/10-1510.1

Biati, A., Karbassi, A.R., Hassani, A.H., Monavari, S.M. & Moattar, F. (2010), "Role of metal species in flocculation rate during estuarine mixing", *International Journal of Environmental Science & Technology*, vol. 7, no. 2, pp. 327-336, DOI: 10.1007/BF03326142

Bighiu, M.A., Gorokhova, E., Almroth, B.C. & Wiklund, A.K.E. (2017), "Metal contamination in harbours impacts life-history traits and metallothionein levels in snails", *PLoS One*, vol. 12, no. 7, p. e0180157, DOI: 10.1371/journal.pone.0180157.

Boyle, E.A., Edmond, J.M. & Sholkovitz, E.R. (1977), "The mechanism of iron removal in estuaries", *Geochimica et Cosmochimica Acta*, vol. 41, no. 9, pp. 1313-1324, DOI: 10.1016/0016-7037(77)90075-8

Davies, C.A., Tomlinson, K. & Stephenson, T., (1991), "Heavy metals in river tees estuary sediments", *Environment Technology*, vol. 12, pp. 961-972.

Davis, J.A. (1984), "Complexation of trace metals by adsorbed natural organic matter", *Geochimica et Cosmochimica Acta*, vol. 48, no. 4, pp. 679-691, DOI: 10.1016/0016-7037(84)90095-4 R.J.

Davutluoglu, O.I., Seckin, G., Ersu, C.B., Yilmaz, T. & Sari, B. (2011), "Heavy metal content and distribution in surface sediments of the Seyhan River, Turkey", *Journal of Environment Management*, vol. 92, no. 9, pp. 2250-2259.

Duggan, M., Bayliss, P., & Burford, M.A. (2019), "Predicting the Impacts of Freshwater-Flow Alterations on Prawn (*Penaeus Merguiensis*) Catches", *Fisheries Research*, vol. 215, pp. 27–37, DOI: 10.1016/j.fishres.2019.02.013

Eliku, T. & Leta, S. (2018), "Spatial and seasonal variation on physico –chemical parameters and heavy metals in Awash River Ethiopia", *Applied Water Science*, vol. 8, no. 6, p. 177, DOI: 10.1007/s13201-018-0803-e

Forstner, U. & Wittmann, G.T.W. (1981), *Metal pollution in aquatic environment*, Springer Verlag, New York, 486 p.

Galkus, A., Joksas, K., Stakeniene, R. & Lagunaviciene, L. (2012), "Heavy Metal Contamination of Harbor Bottom Sediments", *Polish Journal of Environmental Studies*, vol. 21, pp. 1583–1594.

Gibbs, R.J. (1986), "Segregation of metals by coagulation in estuaries", *Marine Chemistry*, vol. 18, no. 2–4, pp. 149-159, DOI: 10.1016/0304-4203(86)90004-6

Gillanders, B.M., Elsdon, T.S., Halliday, I.A., Jenkins, G.P., Robins, J.B., & Valesini, F.J. (2011), "Potential Effects of Climate Change on Australian Estuaries and Fish Utilising Estuaries: a Review", *Marine and Freshwater Research*, vol. 62, pp. 1115–1131, DOI: 10.1071/MF11047

Gu, Y.G., & Gao, Y.P. (2021), "A new method for estimating sedimental integrated toxicity of heavy metal mixtures to aquatic biota: a case study", *Ecotoxicology*, vol. 30, no. 2, pp. 373–380, DOI: 10.1007/s10646-021-02346-0

Guerra-García, J.M., & García-Gómez, J.C. (2004), "Polychaete assemblages and sediment pollution in a harbour with two opposing entrances", *Helgoland Marine Research*, vol. 58, pp. 183–191.

Hang, X., Wang, H., Zhou, J., Du, C. & Chen, X. (2009), "Characteristics and accumulation of heavy metals in sediments originated from an electroplating plant", *Journal of Hazardous Materials*, vol. 163, vol. 2-3, pp. 922-930.

Hassellöv, M. & von der Kammer, F. (2008), "Iron oxides as geochemical nanovectors for metal transport in soil-river systems", *Elements*, vol. 4, no. 6, pp. 401-406, DOI: 10.2113/gselements.4.6.401

Heidari, M. (2019), "Role of natural flocculation in eliminating toxic metals", *Archives of Environmental Contamination and Toxicology*, vol. 76, no. 3, pp. 366-374, DOI: 10.1007/s00244-019-00597-x

Ho, H.H. Swennen, R. & Van Damme, A. (2010), "Distribution and contamination status of heavy metals in estuarine sediments near Cua Ong Harbor, Ha Long Bay, Viatinam", *Geologica Beligica*, vol. 13, no. 1-2, pp. 37-47

Hortellani, M.A., Sarkis, J.E.S., Abessa, D.M.S. & Sousa, E.C.P.M. (2008), "Avaliação da contaminação por elementos metálicos dos sedimentos do estuário Santos – São Vicente", *Química Nova*, vol. 31, no. 1, pp. 10-19.

Iglesias I., Bio A., Bastos L. & Avilez-Valente P. (2021), "Estuarine hydrodynamic patterns and hydrokinetic energy pro-

duction: The Douro estuary case study", *Energy*, vol. 222, p. 119972, DOI: 10.1016/j.energy.2021.119972.

Impellitteri, C.A., Lu, Y., Saxe, J.K., Allen, H.E., & Peijnenburg, W.J.G.M. (2002), "Correlation of the partitioning of dissolved organic matter fractions with the desorption of Cd, Cu, Ni, Pb and Zn from 18 Dutch soils", *Environmental International*, vol. 28, no. 5, pp. 401–410.

Izegaegbe, J., Vivier, L., & Mzimela, H. (2020), "Macrobenthic Community Structure of the Mhlathuze Estuary, a Permanently Open Estuarine Embayment in KwaZulu-Natal, South Africa", *African Journal of Aquatic Science*, vol. 45, no. 1-2, pp. 95–107, DOI: 10.2989/16085914.2020.1719818

Karlsson, T., Persson, P. & Skyllberg, U. (2006), "Complexation of copper(II) in organic soils and in dissolved organic matter - EXAFS evidence for chelate ring structures", *Environmental Science Technology*, vol. 40, pp. 2623-2628

Kotzé, P. (2016), "Protecting Nature's Nurseries - Study Highlights Importance of Natural Flow to Estuaries", The Water Wheel, vol. 15, no. 4, pp. 12–16.

Kulikowska, D., Gusiatin, Z.M., Bulkowska, K. & Kierklo, K. (2015), "Humic substances from sewage sludge compost as washing agent effectively remove Cu and Cd from soil *Chemosphere*, vol. 136, pp. 42-49

Luoma, S.N. (1983), "Bioavailability of trace metals to aquatic organisms — a review", *Science of The Total Environment*, vol. 28, no. 1-3, pp. 1–22, DOI: 10.1016/S0048-9697(83)80004-7

Ma, C., Ci, K., Zhu J., Sun, Z., Liu, Z., Li X., Zhu Y., Tang, C., Wang, P. & Liu Z. (2021), "Impacts of exogenous mineral silicon on cadmium migration and transformation in the soil-rice system and on soil health", *Science of The Total Environment*, vol. 759, p. 143501.

Manceau, A. & Matynia, A. (2010), "The nature of Cu bonding to natural organic matter", Acta Geochimica et Cosmochimica Acta, vol. 74, no. 9, pp. 2556-2580.

Monte, C. do N., Rodrigues, A.P. de C, de Freitas, A.R., Freire, A.S., Santelli, R.E., Braz, B.F. & Machado, W. (2019), "Dredging impact on trace metal behavior in a polluted estuary: a discussion about sampling design", *Brazilian Journal of Oceanography*, vol. 67, DOI: 10.1590/S1679-87592019022706701

Moon, E.M. & Peacock, C.L. (2012), "Adsorption of Cu(II) to ferrihydrite and ferrihydrite-bacteria composites: importance of the carboxyl group for Cu mobility in natural environments", *Geochimia et Cosmochimia Acta*, vol. 92, pp. 203-219.

Nystrand M.I., Österholm P., Nyberg M.E., Gustafsson J.P. (2012), "Metal speciation in rivers affected by enhanced soil erosion and acidity", *Applied Geochemistry*, vol. 27, pp. 906-916, DOI: 10.1016/j.apgeochem.2012.01.009

Okoro, H.K., Fatoki, O.S., Adeloka, F.A., Ximba, B.J. & Synam, R.G. (2012), "A review of sequential extraction procedures for heavy metals speciation in soil and sediments", *Journal of Environmental Anal* Tox, vol. 1, no. 3, pp. 1-9.

Parker J.C. & Rae J.E. (1998), *Environmental Interactions of Clays*, Springer Verlag, Berlin, Heidelberg, New York, 271 p.

Passos, E. de A., Alves, J.C., dos Santos, I.S., Alves, J. do P.H., Garcia, C.A.B. & Spinola Costa, A.C. (2010), "Assessment of trace metals contamination in estuarine sediments using a sequential extraction technique and principal component analysis", *Microchemical Journal*, vol. 96, no. 1, pp. 50-57.

Passos, E.A., Alves, J.P.H., Garcia, C.A.B. & Costa, A.C.S. (2011), "Metal Fractionation in Sediments of the Sergipe River, Northeast, Brazil", *Journal of the Brazilian Chemical Society*, vol. 22, no. 5, pp. 828-835.

Pinto, R., & Marques, J.C. (2015), "Ecosystem Services in Estuarine Systems: Implications for Management," in Chicharo, L., Müller, F. & Fohrer, N., *Ecosystem Services and River Basin Ecohydrology*, Dordrecht, NL, Springer, DOI: 10.1007/978-94-017-9846-4_16

Pokrovsky, O.S. & Schott, J. (2002), "Iron colloids/organic matter associated transport of major and trace elements in small boreal rivers and their estuaries (NW Russia)", *Chemical Geology*, vol. 190, no. 1-4, pp. 141-179, DOI: 10.1016/S0009-2541(02)00115-8

Prabhakaran, P., Ashraf, M. A., & Aqma, W. S. (2016), "Microbial stress response to heavy metals in the environment", *RSC Advances*, vol. 6, no. 111, pp. 109862–109877, DOI: 10.1039/C6RA10966G

Pye, K. & Blott, S.J. (2004), "Particle size analysis of sediments, soils and related particulate materials for forensic purposes using laser granulometry", *Forensic Science International*, vol. 144, no. 1, pp. 19-27.

Qin, C., Yuan, X., Xiong, T., Tan, Y.Z. & Wang, H. (2020), "Physicochemical properties, metal availability and bacterial community structure in heavy metal-polluted soil remediated by montmorillonite-based amendments", *Chemosphere*, vol. 261, p. 128010

Relic, D., Dordevic, D., Popovic, A., Jadranin, M. & Polic, P. (2010), "Fractionation and potential mobility of trace metals in Danube alluvial aquifer within an industrialized zone", *Environmental Monitoring and Assessment*, vol. 171, pp. 229-248.

Rosado, D., Usero, J., & Morillo, J. (2016a). Assessment of heavy metals bioavailability and toxicity toward Vibrio fischeri in sediment of the Huelva estuary", *Chemosphere*, vol. 153, pp. 10–17.

Rosado, D., Usero, J., & Morillo, J. (2016b), "Ability of 3 extraction methods (BCR, Tessier and protease K) to esti-

mate bioavailable metals in sediments from Huelva estuary (Southwestern Spain)", *Marine Pollution Bulltin*, vol. 102, pp. 65–71.

Sahara, E., Widihati, I.A.G & Putra, I.G.D. (2015), "Fraksinasi dan bioavailabilitas logam berat Fe dan Mn pada sedimen di Pelabuhan Benoa", Journal Kimia (Journal of Chemistry), vol. 9, pp. 124–31

Samani, A.R.V., Karbassi, A.R., Fakhraee, M., Heidari, M., Vaezi, A.R. & Valikhani, Z. (2014), "Effect of dissolved organic carbon and salinity on flocculation process of heavy metals during mixing of the Navrud River water with Caspian Seawater", *Desalination Water Treatment*, vol. 55, no. 4, pp. 926-934, DOI: 10.1080/19443994.2014.920730

Sholkovitz, E.R. (1978), "The flocculation of dissolved Fe, Mn, Al, Cu, Ni, Co and Cd during estuarine mixing", *Earth and Planetary Science Letters*, vol. 41, no. 1, pp. 77-86, DOI: 10.1016/0012-821X(78)90043-2

Sholkovitz, E.R., Boyle, E.A. & Price N.B. (1978), "The removal of dissolved humic acids and iron during estuarine mixing", *Earth and Planetary Science Letters*, vol. 40, pp. 130-136, 10.1016/0012-821X(78)90082-1

Silveira, M. L. A., Alleoni, L. R. F., & Guilherme, L.R.G. (2003), "Biosolids and heavy metals in soils", *Scientia Agricola*, vol. 60, no. 4, pp. 793–806. DOI: 10.1590/S0103-90162003000400029

SinghS.K., Subramanian, V. & Gibbs, R.J. (1984), "Hydrous Fe and Mn oxides – scavengers of heavy metals in the aquatic environment", *Critical Reviews in Environmental Control*, vol. 14, no. 1, 1984), pp. 33-90, DOI: 10.1080/10643388409381713

Smith, A., Means, L., Chen, A., Alleman, B., Chapman, C., Tixier Jr., J.S., Brauning, E., Gavaskar, R. & Royer, M.D. (1995), *Remedial Options for Contaminated Sites*, CRC, Boca Raton, FL.

Stolpe, B. & Hassellöv, M. (2007), "Changes in size distribution of fresh water nanoscale colloidal matter and associated elements on mixing with seawater", *Geochimica et Cosmochimica Acta*, vol. 71, no. 13, pp. 3292-3301, DOI: 10.1016/j.gca.2007.04.025

Strawn, D.G. & Baker, L.L. (2009), "Molecular characterization of copper in soils using X-ray absorption spectroscopy", *Environmental Pollution*, vol. 157, no. 10, pp. 2813-2821

Sun, T., Zhang, H., Yang, Z., & Yang, W. (2015), "Environmental Flow Assessments for Transformed Estuaries", *Journal of Hydrology*, vol. 520, pp. 75–84. DOI: 10.1016/j.jhydrol.2014.11.015

Sunda, W.G. & Lewis, J.A.M. (1978), "Effect of complexation by natural organic ligands on the toxicity of copper to unicellular alga, Monochrysis lutheri Limnol", *Limnology and Oceanography*, vol. 23, no 5, pp. 870-876, DOI: 10.4319/lo.1978.23.5.0870

Tarnawski, M., & Baran, A. (2018), "Use of chemical indicators and bioassays in bottom sediment ecological risk assessment", *Archives of Environmental Contamination and Toxicology*, DOI: 10.1007/s00244-018-0513-2.

Turner A., Martino M. & Le Roux, S.M. (2002), "Trace metal distribution coefficients in the Mersey Estuary, UK: evidence for salting out of metal complexes", *Environmental Science & Technology*, vol. 36, no 21, pp. 4578-4584, DOI: 10.1021/es020075y

Vermeiren, P., & Sheaves, M. (2014), "Predictable Habitat Associations of Four Crab Species across the Low Intertidal Landscape of a Tropical Estuary over Time", *Estuaries and Coasts*, vol. 38, no. 1, pp. 285–295, DOI: 10.1007/s12237-014-9799-0

Wang, Z., Wang, Y., Chen, L., Yan, C., Yan, Y., & Chi, Q. (2015), "Assessment of metal contamination in coastal sediments of the Maluan Bay (China) using geochemical indicators and multivariate statistical approaches", *Marine Pollution Bulletin*, vol. 99, pp. 43–53.

Warren, L.A. & Haack, E.A. (2001), "Biogeochemical controls on metal behaviour in freshwater environments", *Earth-Science Reviews*, vol. 54, no. 4, pp. 261-320, DOI: 10.1016/S0012-8252(01)00032-0

Wells, M.L., Smith, G.J. & Bruland, K.W. (2000), "The distribution of colloidal and particulate bioactive metals in Narragansett Bay, RI", *Marine Chemistry*, vol. 71, no. 1-2, pp. 143-163, DOI: 10.1016/S0304-4203(00)00046-3

Wolińska, A., Banach, A., Szafranek-Nakonieczna, A., Stępniewska, Z., & Błaszczyk, M. (2018), "Easily degradable carbon - an indicator of microbial hotspots and soil degradation", *International Agrophysics*, vol. 32, pp. 123–131.

Wu, G., Pan, L., Wei, Q. & Guo, L. (2015), "Decreased mobility of heavy metal in Haihe River sediments: the possible role of tide gate", *Journal of Geochemical Exploration*, vol. 157, pp. 92–99

Wu, Y., Bao, H., Unger, D., Herbeck, L.S., Zhu, Z., Zhang, J., Jennerjahn, T.C. (2013), "Biogeochemical behaviour of organic carbon in a small tropical river and estuary, Hainan, China", Continental Shelf Research, vol. 57, pp. 32–43.

Yi, J., Lo, L.S.H., Liu, H., Qian, P.Y. & Cheng, J. (2021), "Study of Heavy Metals and Microbial Communities in Contaminated Sediments Along an Urban Estuary", *Frontiers in Marine Science*, vol. 8, pp. 741912, DOI: 10.3389/fmars.2021.741912

Zaaboub, N., Oueslati, W., Helali, M.A., Abdeljaouad, S., Huertas, J.F., Galindo, A.L. (2014), "Trace elements in different marine sediment fractions of the Gulf of Tunis (Central Mediterranean Sea)", *Chemical Speciation and Bioavailability*, vol. 26, pp. 1–12.

Zhang, M., Cui, B., Zhang, Z., & Jiang, X. (2016b), "Salinity--oriented Environmental Flows for keystone Species in the Modaomen Estuary, China", *Frontiers of Earth Science*, vol. 11 no. 4, pp. 670–681, DOI: 10.1007/s11707-016-0609-9

Zhong, X.L., Zhou, S.L., Zhu, Q. & Zhao, Q.G. (2011), "Fraction distribution and bioavailability of soil heavy metals in the Yangtze River Delta-a case study of Kunshan City in Jiangsu Province, China", Journal of Hazardous Materials, vol. 198, pp. 13-21.

Zhou, S., Chen, S., Yuan, Y. & Lu, Q. (2015), "Influence of humic acid complexation with metal ions on extracellular electron transfer activity", *Scientific Reports*, vol. 5, no. 1, pp. 1-9.