



Studies on the Contamination of Heavy Metals and Their Chemical Speciation in Sediment from Selected Locations of Pune District

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Nat. Env. & Poll. Tech.
Website: www.neptjournal.com

Received: 27-02-2023

Revised: 11-04-2023

Accepted: 23-04-2023

Key Words:

Heavy metals
Heavy metals speciation
Sequential extraction
Sediment contamination

ABSTRACT

The heavy metal speciation analysis in sediments helps us understand and evaluate essential and unavoidable issues in terms of both health and environmental hazards imposed by these metals in our lives. Analyzing the total content of heavy metals enables us to understand only the quantity of the contaminants. To understand the different species or the chemical forms of heavy metals available in the sediments, we must study their speciation. Speciation studies help us determine their possible sources as well as their environmental stability in terms of availability to plants and other organisms. The heavy metals in this study were specified using four-stage sequential extraction, also known as the BCR technique. This study mainly highlights the quantification of metal contamination of Cu, Zn, Pb, Ni, Cd & Cr, and chemical forms as species in sediment samples collected from different Pune District, Maharashtra sites. Heavy metal contamination from the collected samples was analyzed with the use of flame atomic absorption spectrometry. This study indicated that Zn and Ni are among the most abundant metals in the sediment samples; however, Cu and Cd belong to the least abundant category. The oxidizable and residual forms (immobile and cannot be used by the organisms readily) appeared dominant for most heavy metals. Very significant differences were observed in the speciation of heavy metals from sample to sample, which was probably due to differences in water/soil composition and the agrochemicals like pesticides, weedicides, and fertilizers used in agricultural practices; the wastewater generated from different pharmaceuticals, chemical processing and manufacturing industries as well as the improper wastewater treatment methods.

INTRODUCTION

Heavy metals contribute to the vast spectrum of pollutants, causing chronic impacts on human health and the ecosystem. Various practices such as agricultural, medical, industrial, domestic, medical, and production-based technological activities result in the wide dispersion of heavy metals within the environment. Studying the impact of these contaminants on human health and the immune system has become an area of great interest among scholars due to their wide range of distribution and their careless use in most industries and our day-to-day lives (Guan et al. 2001, Yan et al. 2008, Christoforidis & Stamatis 2009). Heavy metals have earned the fame of being dreadful environmental pollutants due to their toxic ability to cause huge disruption and discord regarding nutritional, ecological, and environmental balances (Goyer & Clarkson 1996, Wang & Shi 2001). Numerous factors determine the toxicity of heavy metals, including the chemical type, source of contamination, the contaminant's distribution route, and the recipient's age, sex, genetics, and immunity or nutritional status.

As per the current global condition, arsenic, cadmium, chromium, mercury, and lead are on the list of the heavy metals studied due to their very high toxicity causing great worry for the public health sector. The study of heavy metal contamination of soil and sediments helps assess their quality and degree of contamination (Su & Wong 2004, Walter et al. 2006). The bioavailability and mobility of these contaminants depend on their species or chemical forms (Fuentes et al. 2004, Wang et al. 2006). The method commonly used to study the speciation of these metals is the three-step sequential extraction procedure, also known as the BCR technique (Community Bureau of Reference), a modified version of the Tessier method developed in 1979 (Tessier et al. 1979, Rauret et al. 2000, Álvarez et al. 2002). The commonly found heavy metals in wastewater include -Arsenic, Cadmium, Chromium, Copper, Lead, Nickel, and Zinc, which results in serious human health and environmental disruption by obtaining easy access to the ecosystem by both natural and man-made activities. (Beyersmann & Hartwig 2008). The wide distribution of these contaminants into the ecosystem ranges from natural

activities like soil erosion, and weathering of rocks to man-made activities like mining, industrial run-offs, sewage discharge, agricultural practices of using insecticides and pesticides, and so on (Arruti et al. 2010).

The procedure allows us to determine the contaminants in the soil and sediment samples, particularly the species in the bound form that can readily migrate from the sediment to the soil and then further become a part of the ecosystem. (Sutherland 2010, Rashed et al. 2011). The study's core objectives were to first analyze heavy metal concentration present in the water, soil, and sediment samples from different locations of Pune District in Maharashtra. Secondly, to investigate the species or chemical forms of the contaminants in the sediment samples from selected seven sites of Pune District using the BCR sequential extraction technique, which focuses on evaluating their bioavailability and mobility. Finally, to determine the quality of the soil and sediment for agrarian practices based on the degree of contamination by heavy metals in samples and their degree of toxicity.

MATERIALS AND METHODS

Total Content of Heavy Metals in Sediment

The sample preparation comprised air drying for 24 h at 24°C. Afterward, the sediment was crushed into a fine powder and subjected to digestion. A conventional aqua regia digestion technique was employed for this purpose. Firstly, a homogeneously mixed sample of 0.5 g was shifted to a clean 250 mL glass beaker. The sample was digested on a hotplate for 3 h using 12 mL of aqua regia at 110°C. Once the sample was observed to have obtained near dryness consistency, it was reduced using 20 mL -Nitric Acid (2 % v/v, H₂O) & collected in a 100 mL flask, later filtering through Whatman 42 filter paper.

Further, it was watered down using 100 mL of deionized water. The total heavy metal content in the samples was analyzed using an Atomic Absorption Spectrometer, employing SpectraAA 220, VARIAN. When the metal concentration was observed to obtain a standard curve., Samples were further diluted with deionized water. All the assessments were carried out thrice for the sake of accuracy.

Speciation of the Heavy Metals in Sediment

Sequential extraction of heavy metals in sediment samples: The speciation study was performed by the three-stage extraction, the modified BCR technique proposed by the European Community Bureau of Reference, which facilitates the evaluation of metal species present in the sample.

Fraction 1 – Bound to Carbonates (Soluble in acids) – These are extracted using Acetic Acid (CH₃COOH) – applicable to bound to carbonates form that can readily pass to the soil and are among the most readily available forms, where they are bioavailable and migrates to water bodies very easily (transportable).

Fraction 2 – Attached to Manganese and Ferrous oxides (Reducible) – These are concentrated using hydroxylamine hydrochloride (NH₂OH-HCl) – attached to Iron and Manganese oxide forms. Migration capacity and absorption are slower than Fraction 1 (transportable) forms.

Fraction 3 – Attached to organic matter and sulfides (Oxidizable) – These are concentrated using hydrogen peroxide (H₂O₂)– applicable to bound to organic matter and sulfide forms. They are neither readily absorbed nor have the migration capacity as the forms observed in Fraction 1 and Fraction 2 (non-transportable).

Fraction 4 – Residual Fraction – These are concentrated using aqua-regia (3 parts of HCl and 1 part of HNO₃) – applicable to the residual metal concentrate obtained after extraction from the previous fractions. They are practically inaccessible to the plants and hence have no capacity to migrate (non-transportable).

BCR procedure: The sample preparation for the metal speciation involved drying the sediment sample (48 h at 20°C, air-dried to obtain constant mass at 105°C) followed by grinding to obtain a fine powder of homogeneously mixed sample. The sequential extraction of the heavy metal fractions of sediment samples was performed using the following steps:

Step 1 – Fraction 1: Bound to Carbonates (Extraction of exchangeable fraction):

1 g of a homogeneously mixed dried sample was digested by adding 40 mL, 0.11 mol acetic acid, in a 100 cm³ centrifuge tube. This tube was stirred for 16 h at normal temperature, at 30 rpm, and then centrifuged at 3000 rpm for 20 min. The supernatant was obtained, then stored aside for testing. The leftover remainder was washed with 5 mL of deionized distilled water and centrifuged again. The supernatant was then discarded. This leftover residue was kept aside to be used in Step 2.

Step 2 – Fraction 2: Attached to Mn and Fe Oxides (Reducible fraction):

40 mL 0.1 M hydroxylamine hydrochloride was added to the residue obtained in the first Step. This tube was again agitated for 16 hrs. at 24°C, at 30 rpm, and centrifuged later at 3000 rpm (20 min.). The supernatant obtained was stored aside for analysis. The leftover residue was washed with 5

mL of deionized distilled water and centrifuged again. The supernatant was discarded, and the leftover residue was kept aside for use in the next step.

Step 3 – Fraction 3: Attached to Sulfides and Organic matters (Extraction of Oxidizable Form):

10 mL of 30% H₂O₂ was carefully added to the residue obtained from the previous extraction stage, transferred to transparent glassware, and left for 11 h at normal temperature with occasional stirring. The sample was then incubated at 85°C for 1 h. The residue was combined with 50 mL of Ammonium acetate and centrifuged at 3000 rpm for 20 min.). The supernatant was stored aside for analysis in a separate polyethylene container, followed by the procedure conducted in Step 1 and Step 2.

Step 4 – Fraction 4: Extraction of Residual Fraction with aqua regia (mixture of 3 parts HCl + 1-part HNO₃):

5 mL of HNO₃ & 15 mL of HCl were mixed with residue obtained from the previous stage, then evaporated to dryness. On chilling, 5% of HNO₃ was used to dissolve the residue. The metal concentrations were analyzed using Flame Atomic Absorption Spectrometry, SpektraAA 220 VARIAN. All of the analyses were repeated thrice for the sake of accuracy.

RESULTS AND DISCUSSION

Physicochemical Properties of the Sediment Samples

Table 1 indicates that on examining the sediment samples for the heavy metal contaminants, namely, Cd, Cr, Cu, Co, Fe, Mn, Mg, Ni, Pb, and Zn, it was clearly found that almost all the heavy metals appeared to be above the permissible limits (WHO, ICMR, BIS) ranging from moderately toxic to highly toxic. We also observed that the levels of heavy metals increase as we move from the upstream to the central and the downstream regions. This table represents the total concentration of these metals in the sediment samples from

different locations in Pune.

As per the available research data, the concentration of these metal contaminants present in the sewage sludge can be arranged as follows Zn > Cu > Cr > Ni > Pb > Cd (this order does not include Mn, Mg, Fe, Co) (Shrivastava & Banerjee 2004). The order we obtained after examining the Sediment Samples Ni > Mn > Pb > Cd > Cr > Cu > Zn > Co. In this study, we notice that the Ni ranges from 30 to 50 times more than the permissible limits. Likewise, Pb ranges more than 30 times; Mn more than 33 times; Cd more than 10 times; and Cr more than 4 times the permissible limits suggested by the World Health Organisation (WHO 2019).

Table 2 gives us an idea of the heavy metal speciation results of selected metals from Sediment samples from different sites in the Pune District. Fig. 1 explains the Zn Speciation (Heavy metal speciation) of sediment samples from Site 1 to Site 7 (Fraction 1 to Fraction 4). In this case, Fraction 3 (F-3) results appear to be in higher concentration than F-1, F-2 and F-3.

Fig. 2 describes the Cu Speciation of sediment samples from Site 1 to Site 7 (Fraction 1 to Fraction 4). Here, Fraction 3 outcomes indicate that F-3 exhibits higher concentration than F-1, F-2, and F-4.

Fig. 3 and Fig. 4 report about the concentration of Ni and Pb Speciation of sediment samples from Site 1 to Site 7 (Fraction 1 to Fraction 4). As far as Ni speciation is concerned, Fractions 1 and 2 seem to be the lowest, however, F-3 and F-4 indicate a slight increase than the former two. However, while studying the Pb speciation it is clearly understood that F-3 and F-4 species of the heavy metal is present in way higher concentration than F-1 and F-2. The arrangement of concentration of Pb metal species could be better explained like F-3 > F-4 > F-1 > F-2.

Table 1: Heavy metal analysis of sediment samples (upstream to downstream).

Heavy Metals	Site-1	Site-2	Site-3	Site-4	Site-5	Site-6	Site-7
Cd	0.007	0.005	0.014	0.013	0.135	0.029	0.007
Cr	0.027	0.044	0.048	0.002	0.162	1.076	0.047
Cu	0.057	0.059	1.137	0.461	2.6	2.516	0.265
Co	0	0.002	0.572	0.222	0.328	1.031	0.274
Fe	0.004	0.012	111.6	45.6	489.6	715.3	113.4
Mn	0.015	0.008	18.969	4.563	11.502	22.94	11.738
Mg	1.661	13.487	81.72	51.41	71.66	40.58	15.82
Ni	1.14	0.488	2.448	2.625	5.57	15.11	1.954
Pb	0.13	0.09	0.2	0.34	0.72	0.62	0.22
Zn	0.009	0.016	1.181	0.76	2.556	3.471	0.607

Table 2: Heavy metal speciation results of selected metals from sediment samples

Zn	S-1	S-2	S-3	S-4	S-5	S-6	S-7
F-1	0.003	0.005	0.235	0.236	0.025	0.876	0.142
F-2	0.004	0.006	0.096	0.315	0.621	0.93	0.203
F-3	0.005	0.004	1.035	0.325	1.59	1.23	0.29
F-4	0.001	0.003	0.181	0.124	0.325	0.423	0.216
Cu	S-1	S-2	S-3	S-4	S-5	S-6	S-7
F-1	0.014	0.019	0.26	0.128	0.23	0.56	0.032
F-2	0.011	0.02	0.115	0.132	0.051	0.146	0.052
F-3	0.019	0.023	0.45	0.15	1.268	1.058	0.125
F-4	0.012	0.009	0.232	0.145	0.751	0.752	0.056
Ni	S-1	S-2	S-3	S-4	S-5	S-6	S-7
F-1	0.13	0.123	0.22	0.766	1.523	3.708	0.212
F-2	0.023	0.056	0.048	0.042	0.326	0.19	0.025
F-3	0.685	0.266	1.762	1.525	2.322	9.093	1.329
F-4	0.0423	0.045	0.448	0.128	1.59	2.113	0.389
Pb	S-1	S-2	S-3	S-4	S-5	S-6	S-7
F-1	0.012	0.015	0.07	0.065	0.052	0.092	0.02
F-2	0.006	0.023	0.02	0.0423	0.023	0.021	0.01
F-3	0.052	0.042	0.09	0.142	0.422	0.321	0.152
F-4	0.048	0.021	0.06	0.071	0.232	0.215	0.04
Cd	S-1	S-2	S-3	S-4	S-5	S-6	S-7
F-1	0	0.001	0.001	0.001	0.007	0.023	0.001
F-2	0.001	0.003	0.002	0.003	0.009	0.062	0.001
F-3	0.002	0.002	0.006	0.007	0.092	0.177	0.004
F-4	0.004	0.001	0.004	0.002	0.032	0.048	0.002
Cr	S-1	S-2	S-3	S-4	S-5	S-6	S-7
F-1	0.005	0.005	0.007	0	0.023	0.009	0.011
F-2	0.006	0.022	0.008	0.001	0.011	0.056	0.008
F-3	0.009	0.009	0.011	0.0015	0.087	0.988	0.012
F-4	0.007	0.012	0.023	0.0011	0.033	0.087	0.018

Note: Results are expressed in mean mg.kg⁻¹ (PPM); Each sample has been run thrice for accuracy.

Similarly, Fig. 5 and Fig. 6 explain the concentration of Cd and Cr Speciation of sediment samples from Site 1 to Site 7 (Fraction 1 to Fraction 4). Both Cd and Cr exhibit similar results, where F-1, F-2 and F-4 are noted as having lower concentration and F-3 represents higher concentration in the case of both heavy metals.

The obtained results of heavy metal speciation have been mentioned in the table above. The basic aim of the speciation analysis of the sediment samples was to analyze the bioavailability and migration capacity of these contaminants to the water and soil environment to assess their possible

impacts directly or indirectly on the environment and the ecosystem. The total Concentration of the heavy metals at different sites was recorded as follows:

Site 1- Ni > Pb > Zn > Cr > Cu > Cd
 Site 2- Ni > Zn > Cu > Pb > Cd > Cr
 Site 3- Ni > Cu > Pb > Zn > Cd > Cr
 Site 4 – Ni > Zn > Pb > Cu > Cd > Cr
 Site 5 – Ni > Zn > Cu > Pb > Cd > Cr
 Site 6 – Ni > Zn > Pb > Cu > Cr > Cd
 Site 7 – Ni > Zn > Pb > Cu > Cr > Cd

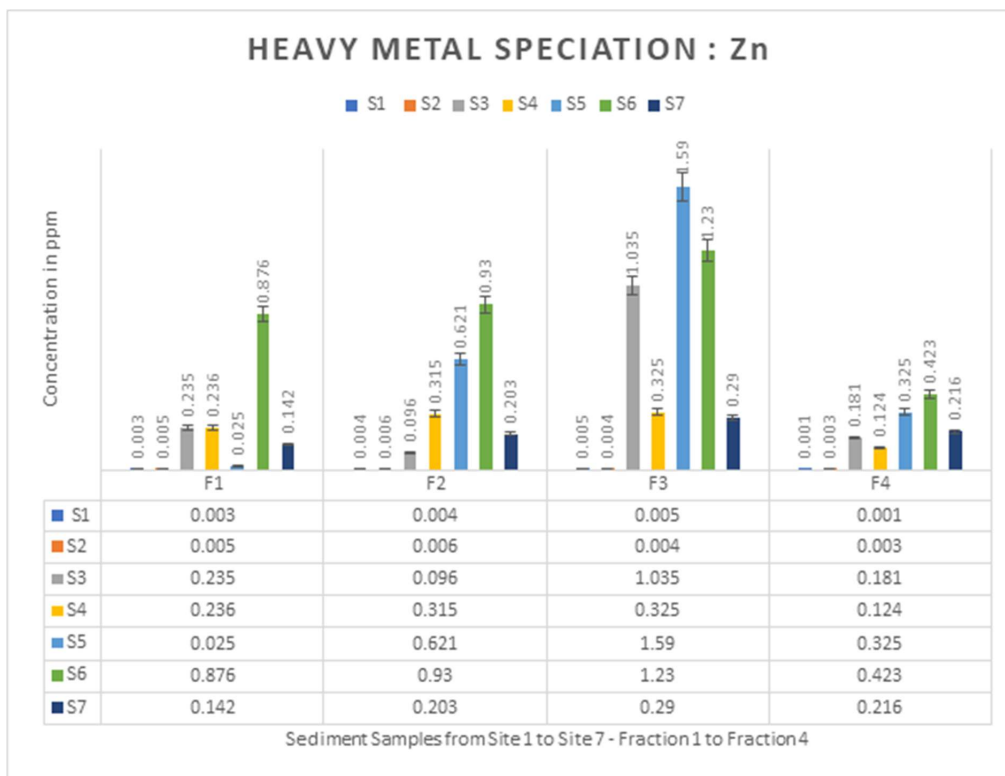


Fig. 1: Heavy metal (Zn) speciation of sediment samples from Site 1 to Site 7 (F1 to F4).

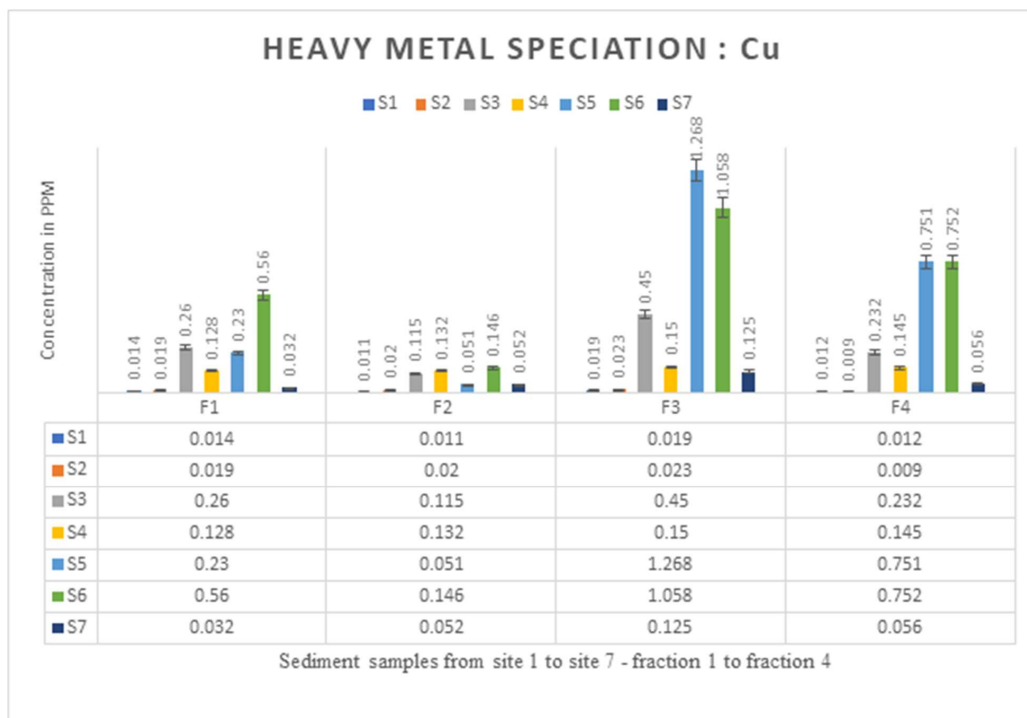


Fig. 2: Heavy metal (Cu) speciation of sediment samples from Site 1 to Site 7 (F1 to F4).

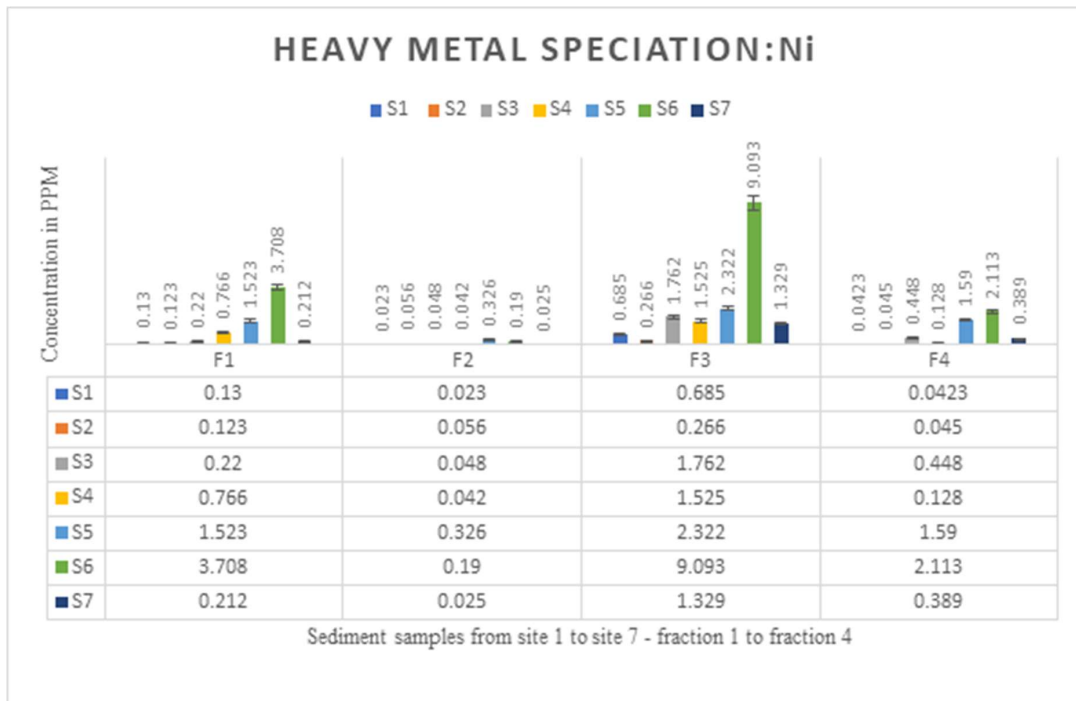


Fig. 3: Heavy metal (Ni) speciation of sediment samples from Site 1 to Site 7 (F1 to F4).

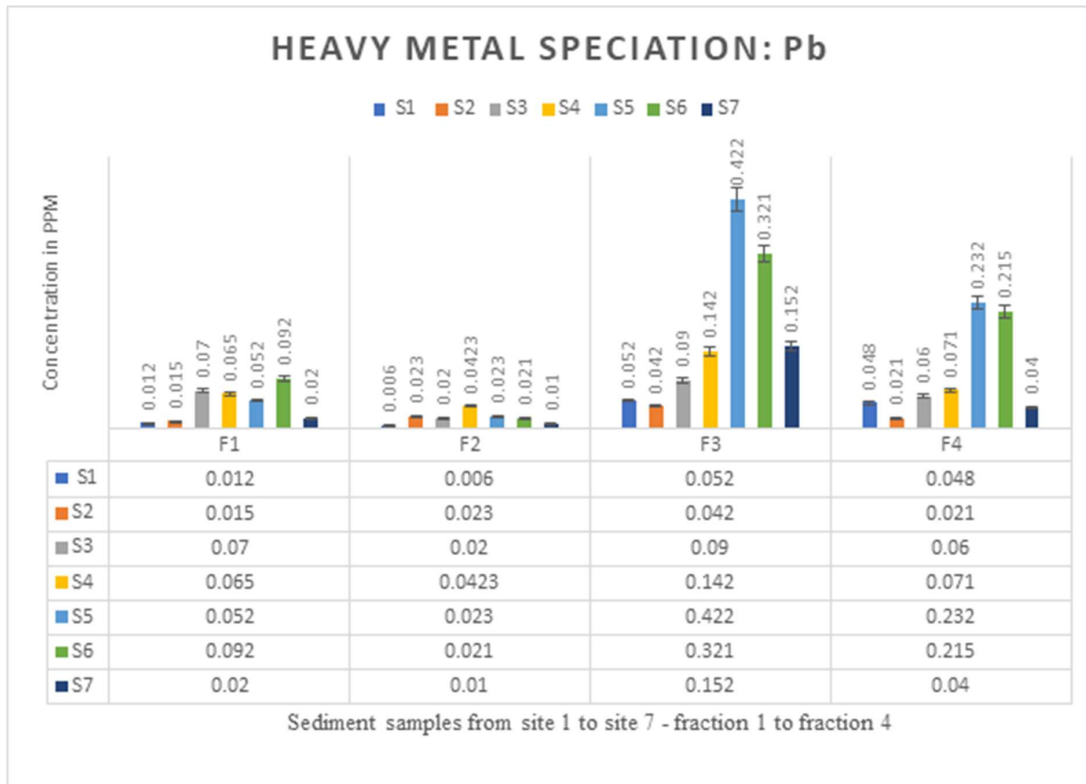


Fig. 4: Heavy metal (Pb) speciation of sediment samples from Site 1 to Site 7 (F1 to F4).

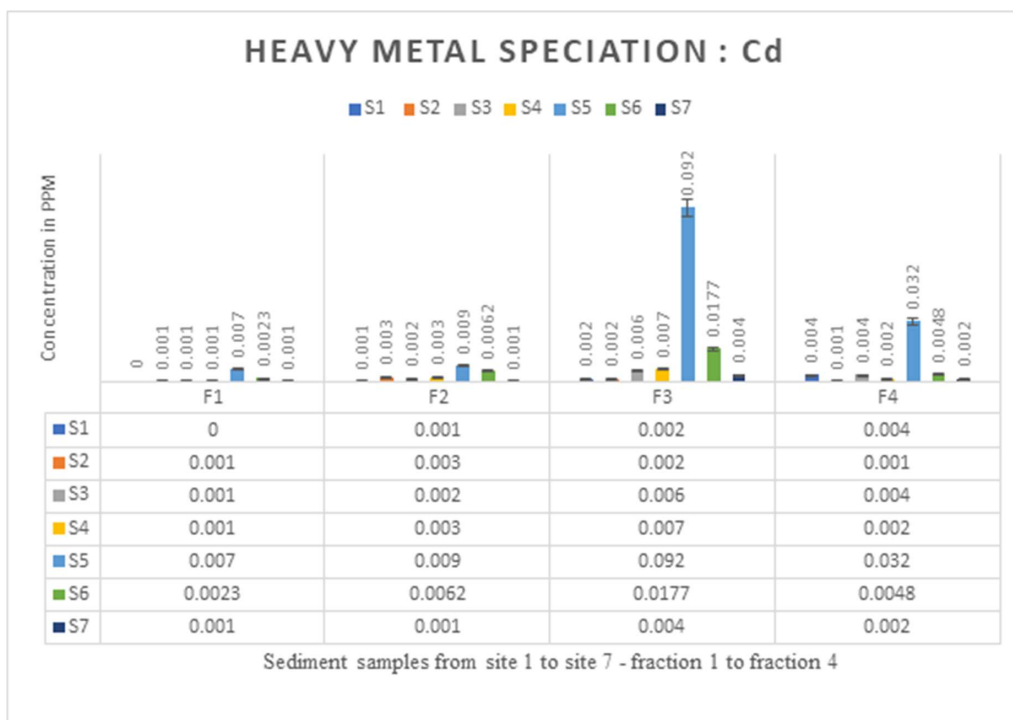


Fig. 5: Heavy metal (Cd) speciation of sediment samples from Site 1 to Site 7 (F1 to F4).

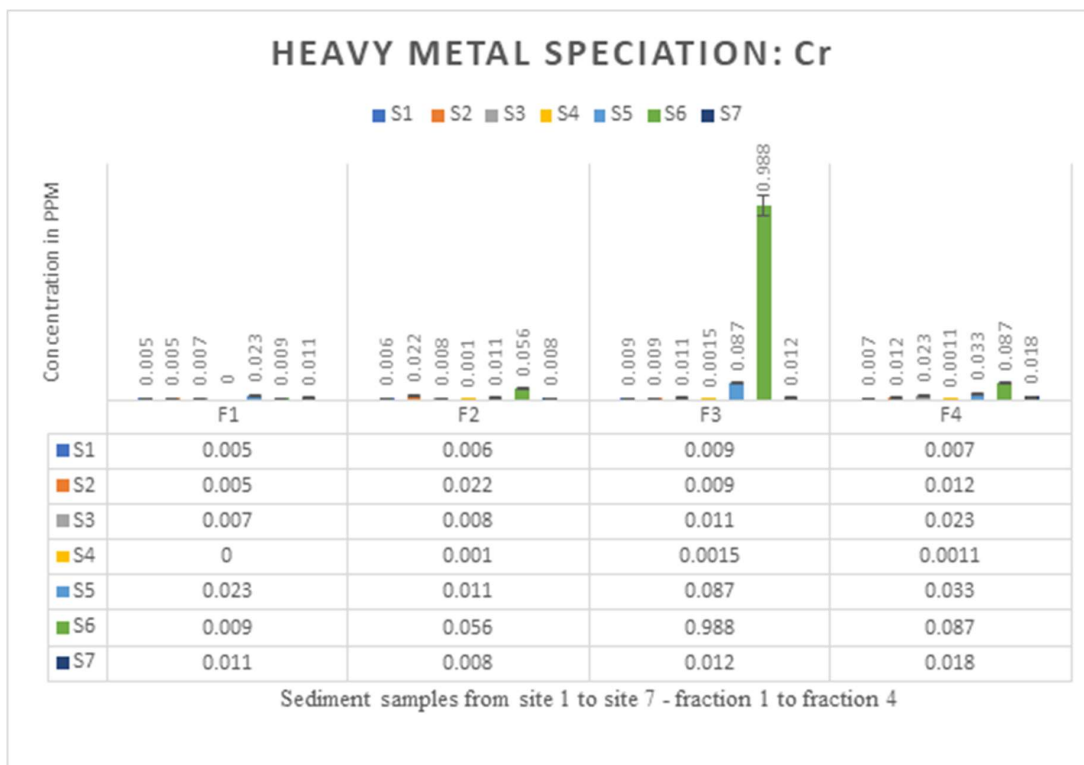


Fig. 6: Heavy metal (Cr) speciation of sediment samples from Site 1 to Site 7 (F1 to F4).

Considering the heavy metal distribution in the selected fractions (F1, F2, F3 and F4), it was recorded as follows:

Site 1 – Ni (F-3 > F-4 > F-1 > F-2), Pb (F-3 > F-4 > F-2 > F-1), Zn (F-3 > F-2 > F-1 > F-4), Cr (F-3 > F-4 > F-2 > F-1), Cu (F-3 > F-1 > F-4 > F-2), Cd (F-4 > F-3 > F-2 > F-1)

Site 2 – Ni (F-3 > F-1 > F-2 > F-4), Zn (F-2 > F-1 > F-3 > F-4), Cu (F-3 > F-2 > F-1 > F-4), Pb (F-3 > F-2 > F-4 > F-1), Cd (F-2 > F-3 > F-1 > F-4), Cr (F-2 > F-4 > F-3 > F-1)

Site 3 – Ni (F-3 > F-4 > F-1 > F-2), Cu (F-3 > F-1 > F-4 > F-2), Pb (F-3 > F-1 > F-4 > F-2), Zn (F-3 > F-2 > F-1 > F-4), Cd (F-3 > F-4 > F-2 > F-1), Cr (F-4 > F-3 > F-1 > F-2)

Site 4 – Ni (F-3 > F-1 > F-4 > F-2), Zn (F-3 > F-2 > F-1 > F-4), Pb (F-3 > F-4 > F-1 > F-2), Cu (F-3 > F-4 > F-2 > F-1), Cd (F-3 > F-2 > F-4 > F-1), Cr (F-3 > F-4 > F-2 > F-1)

Site 5 – Ni (F-3 > F-4 > F-1 > F-2), Zn (F-3 > F-2 > F-4 > F-1), Cu (F-3 > F-4 > F-1 > F-2), Pb (F-3 > F-4 > F-1 > F-2), Cd (F-3 > F-4 > F-2 > F-1), Cr (F-3 > F-4 > F-1 > F-2)

Site 6 – Ni (F-3 > F-1 > F-4 > F-2), Zn (F-3 > F-2 > F-1 > F-4), Pb (F-3 > F-4 > F-1 > F-2), Cu (F-3 > F-4 > F-1 > F-2), Cr (F-3 > F-4 > F-2 > F-1), Cd (F-3 > F-2 > F-4 > F-1)

Site 7 – Ni (F-3 > F-4 > F-1 > F-2), Zn (F-3 > F-4 > F-2 > F-1), Pb (F-3 > F-4 > F-1 > F-2), Cu (F-3 > F-4 > F-2 > F-1), Cr (F-4 > F-3 > F-1 > F-2), Cd (F-3 > F-4 > F-2 > F-1).

Most sediment samples showed high concentrations of heavy metals species attached to residual and oxidizable

and fractions (F-3 and F-4). Fractions 3 and 4 are immobile. Hence they are not readily absorbed by the Plants and move in the food chain from one trophic level to another. Among the mobile forms (F-1 and F-2), F-2 is a reducible form bound to Mn and Fe oxides is much higher than F-1. Other researchers observed similar relations on sludge (Jamali et al. 2007). Fractions 1 and 2 are mobile, meaning they can be quickly utilized by the producers (Plants) and have the capacity to move up in the food chain by leaping from one trophic level to the other as efficiently as possible, intoxicating the organisms that come under its influence directly or indirectly.

The distribution of the heavy metals (Fig. 7) was observed to be highest at the following sites: Zn at Site 1, Cu at Site 4, Ni at Site 5, Pb at Site 2, Cd at Site 2, and Cr at Site 2. However, the lowest distribution was recorded – Zn at Site 6, Cu at Site 5, Ni at Site 1, Pb at Site 1, Cd at Site 3, and Cr at Site 5. It should be noted that results could be impacted by factors such as diverse sample consistency, chemicals used, and the processes or changes taking place while the sample was being analyzed.

Recovery Rate

Table 2 describes the Heavy metal speciation results of the sediments obtained from different locations in the Pune district. The following mathematical operation did verification of the results.

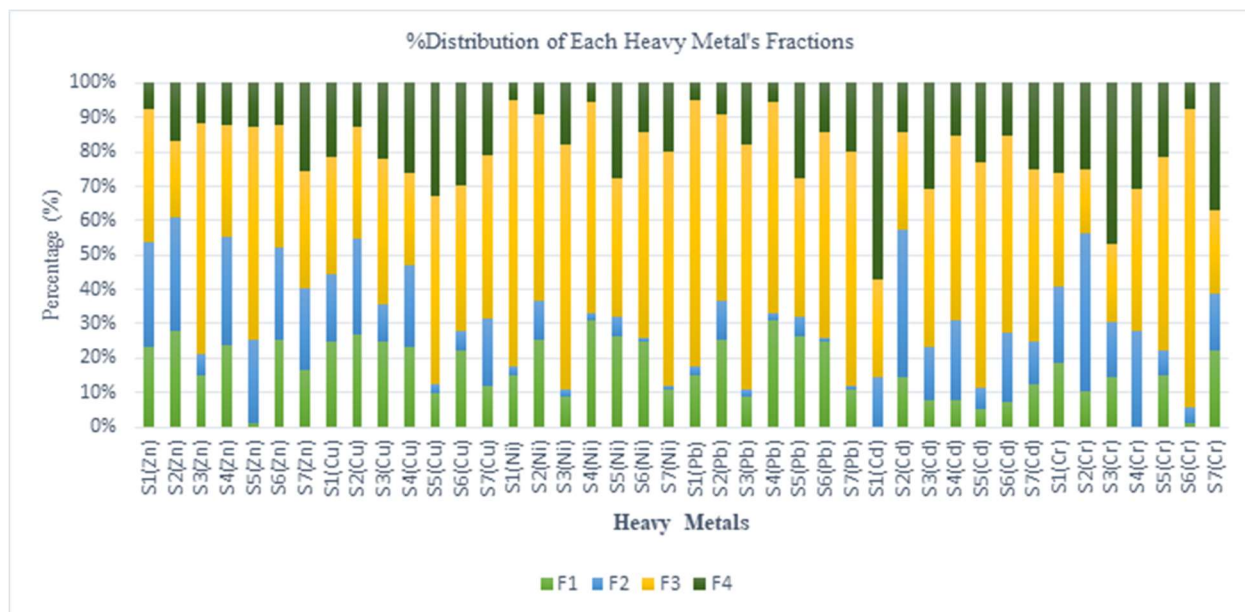


Fig. 7: % Distribution of each metal's fraction in sediment samples from Site 1 to 7.

Table 3: Recovery Rates of the Heavy Metals of the Sediment Samples.

Heavy Metal	S1	S2	S3	S4	S5	S6	S7
Zn	144.4	112.5	130.99	131.57	100.19	99.65	140.19
Cu	98.24	120.33	92.96	120.39	88.46	100	100
Ni	77.21	100.40	101.22	93.75	103.42	99.96	100.05
Pb	90.76	112.22	120	94.20	101.25	104.67	100.90
Cd	100	140	92.85	100	103.70	106.89	114.28
Cr	100	109.09	102.08	180	95.06	105.94	104.25

$$R = [(F-1) + (F-2) + (F-3) + (F-4)]/TC \times 100\%$$

where,

R is the Recovery percentage of the heavy metal

TC (mg.kg⁻¹) is the total concentration of heavy metals

F-1, F-2, F-3 & F-4 are the concentration of heavy metals concentrated in individual fractions.

Here, Table 3 depicts the recovery rates of six heavy metals namely Zn, Cu, Ni, Pb, Cd, and Cr from the sediment samples from different sites of Pune District. The recovery rate of Zn ranged from 99.65 % to 144.44 % for Sediment Samples from Site 1 to Site 7. Similarly, the recovery rate of Cu and Ni was observed between 88.46% to 120.39 % and 77.21 % to 103.42 %, respectively. Likewise, Pb, Cd, and Cr recovery rates ranged from 90.76% to 120.0 %, 92.85 % to 114.28%, and 95.06% to 109.09%, respectively.

CONCLUSION

The total heavy metal concentration investigation was conducted to assess the total concentration of selected heavy metals in sediment samples collected from different locations of Pune (Upstream region to the Downstream Region). Nickel and lead were observed to be among the highest concentrations, while Zinc and Copper were of the lowest ones. In total, the sediment samples from the central part of Pune and the downstream regions (Ujani Backwaters) were recorded to have the highest concentration of heavy metals (Site 5 to Site 7). The heavy metal speciation study indicates that Ni and Zn are among the abundant heavy metals in sediment samples, while Cd and Cr are among the lowest concentrations. In the speciation study, the oxidizable and residual species were found to be leading for all heavy metals. A notable change in the speciation fashion was observed among the sediment samples from different sample collection sites. This could be due to differences in water/soil composition and the agrochemicals like pesticides, weedicides, and fertilizers used in agricultural practices, the wastewater generated from different pharmaceutical industries, chemical processing, and manufacturing industries, as well as the improper wastewater treatment methods.

REFERENCES

- Álvarez, E.A., Mochon, M.C., Sánchez, J.J. and Rodríguez, M.T. 2002. Heavy metal extractable forms in sludge from wastewater treatment plants. *Chemosphere*, 47(7): 765-775.
- Arruti, A., Fernández-Olmo, I. and Irabien, Á. 2010. Evaluation of the contribution of local sources to trace metals levels in urban PM_{2.5} and PM₁₀ in the Cantabria region (Northern Spain). *J. Environ. Monit.*, 12(7): 1451-1458.
- Beyersmann, D. and Hartwig, A. 2008. Carcinogenic metal compounds: recent insight into molecular and cellular mechanisms. *Archives of toxicology*, 82: 493-512.
- Christoforidis, A. and Stamatis, N. 2009. Heavy metal contamination in street dust and roadside soil along the major national road in Kavala's region. *Greece. Geoderma*, 151(3-4): 257-263.
- Fuentes, A., Lloréns, M., Sáez, J., Soler, A., Aguilar, M.I., Ortuño, J.F. and Meseguer, V.F., 2004. Simple and sequential extractions of heavy metals from different sewage sludges. *Chemosphere*, 54(8): 1039-1047.
- Goyer, R.A. and Clarkson, T.W. 1996. *Toxic effects of metals*. The McGraw-Hill Company, NY.
- Guan, D.S., Chen, Y.J. and Ruan, G.D. 2001. Study on heavy metal concentrations and the impact of human activity on them in urban and suburb soils of Guangzhou. *Acta Sci. Nat. Univ. Sunyatseni*, 40(4): 93-6.
- Jamali, M.K., Kazi, T.G., Afridi, H.I., Arain, M.B., Jalbani, N. and Memon, A.R. 2007. Speciation of heavy metals in untreated domestic wastewater sludge by time saving BCR sequential extraction method. *J. Environ. Sci. Health Part A*, 42(5): 649-659.
- Klaassen, C.D., Casarett, L.J. and Amdur, M.O. 1986. *Toxicology: The Basic Science of Poisons*. Macmillan, NY.
- Rashed, M.N., Soltan, M.E., Fawzey, E.M. and El-Taher, M.A. 2011. Impact of sewage sludge spreading on heavy metal speciation in ecosystem. *Indian J. Environ. Protect.*, 1: 37-44.
- Rauret, G., López-Sánchez, J.F., Sahuquillo, A., Barahona, E., Lachica, M., Ure, A.M., Davidson, C.M., Gomez, A., Lück, D., Bacon, J. and Yli-Halla, M. 2000. Application of a modified BCR sequential extraction (three-step) procedure for the determination of extractable trace metal contents in a sewage sludge amended soil reference material (CRM 483), complemented by a three-year stability study of acetic acid and EDTA extractable metal content. *J. Environ. Monit.*, 2(3): 228-233.
- Shrivastava, S.K. and Banerjee, D.K. 2004. Speciation of metals in sewage sludge and sludge-amended soils. *Water, Air, and Soil Pollution*, 152: 219-232.
- Su, D.C. and Wong, J.W.C. 2004. Chemical speciation and phytoavailability of Zn, Cu, Ni, and Cd in soil amended with fly ash-stabilized sewage sludge. *Environ. Int.*, 7(29): 895-900.
- Sutherland, R.A. 2010. BCR@-701: A review of 10-years of sequential extraction analyses. *Anal. Chim. Acta*, 680(1-2): 10-20.
- Tessier, A.P.G.C., Campbell, P.G. and Bisson, M.J.A.C. 1979. Sequential extraction procedure for the speciation of particulate trace metals. *Anal. Chem.*, 51(7): 844-851.

- Walter, I., Martinez, F. and Cala, V. 2006. Heavy metal speciation and phytotoxic effects of three representative sewage sludges for agricultural uses. *Environ. Pollut.*, 3)139): 507-514.
- Wang, C., Li, X.C., Ma, H.T., Qian, J. and Zhai, J.B. 2006. Distribution of extractable fractions of heavy metals in sludge during the wastewater treatment process. *J. Hazard. Mater.*, 137(3): 1277-1283.
- Wang, S. and Shi, X. 2001. Molecular mechanisms of metal toxicity and carcinogenesis. *Mol. Cell. Biochem.*, 222: 3-9.
- Yan, J., Ye, Z.X., Yan, Y. and Huang, X.P. 2008. Study on heavy metals distribution in atmospheric particulate matter on both sides of the Cheng-Ya Expressway. *Sichuan Environ.*, 2)2): 19-21.

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