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Original Research Article

Phytoremediation of Heavy Metals from Municipal Waste Water by *Salvinia molesta* Mitchell

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Abstract: The present study was undertaken to investigate the heavy metal decontaminating activity of *Salvinia molesta* from municipal waste water. The waste water was treated with the plant specimen for five and ten days consecutively after which it was analyzed for the estimation of cadmium (Cd), copper (Cu), iron (Fe), nickel (Ni), lead (Pb) and zinc (Zn) by inductively coupled plasma atomic emission spectroscopy (ICP-AES). The waste water was also analyzed before the treatment. The results of the investigation showed that the non-treated samples indicated higher content of Fe followed by Zn in sample Nitta gelatin (NG). It was found that Cu and Ni were present in similar quantities in all samples. A favorable decrease was seen in Zn in all samples and Fe in sample NG after five days of treatment. The concentration of Cu and Fe were found to be below the detection limit in samples collected from Chalakudy (CH), Ernakulam (ER) and Kokkala (KK) after ten days of treatment. Ni was found to be below the detection limit in samples ER and KK. Zn was found to be at a reduced level in samples CH, ER, KK and NG. Cd and Pb were found to be below the detection limit (0.01 and 0.05 ppm) in all four samples before treatment, after five and ten days of treatment. The present findings revealed that *S. molesta* was efficient in reducing the heavy metal concentration in the waste water, suggesting being a bioaccumulator of these metals.
Keywords: phytoremediation, heavy metals, biomagnification, bioaccumulation, food chain, eco-friendly.

INTRODUCTION

Major water bodies in most of the countries are polluted by the discharge of industrial effluents, domestic sewage and trade waste water containing heavy metals. The environmental pollution with toxic metals has become a worldwide crisis, affecting agriculture and contributing to bioaccumulation and biomagnification in the food chain [1]. It has become a serious global concern and most of the countries around the world are employing low cost and eco-friendly green technology involving plants to decontaminate waste water for a sustainable future [2, 3] Recently scientists have recognized that certain toxic metals may stay on in the environment for a longer time and can eventually bioaccumulate to higher levels that could affect the health of humans, animals and plants [1].

Waste water discharges are causing eutrophication and water borne diseases. The treatment of waste water using phytoremediation is one of the desirable treatment technologies in many parts of the world. This system has the potential to be one of the sustainable solutions for treating and discharging the large quantity of waste water and getting access to safer potable water [4, 5]. The cleanup of most of the contaminated sites is necessary in order to reclaim the area and to reduce the entry of toxic metals into the food chain. Various methods such as soil excavation, soil washing or burning or pumping and treating systems are already being implemented to decontaminate the polluted soil and water. However these techniques are not fully acceptable as they destroy biotic components of soil and water, and are highly expensive to implement. For the last few decades phytoremediation (bioremediation) has emerged as the low cost and effective technology that utilizes the plants and their associated microbial flora for environmental cleanups [6, 7]. Phytoremediation essentially comprises six types of strategies such as phytoextraction, rhizofiltration (phytofiltration), phytostimulation (rhizodegradation), phytovolatilization, phytostabilization (phytoimmobilization) and phytodegradation (phytotransformation) though more than one may be used by the plant simultaneously [8, 9].

Phytoremediation takes advantage of the unique, selective and naturally occurring uptake capabilities of plant root systems together with the translocation, bio-accumulation and pollutant degradation abilities of the entire plant body [10, 11]. According to Hartman [12], plants have been used for the decontamination of waste water for over 300 years. Several plant species have been identified with the capacity to sequester toxic metals in waste water and soil [13]. Aquatic macrophytes play an essential role in heavy metal cycling in the wetlands due to uptake, storage and release processes [14]. Several studies have reported that the aquatic plants are very effective in removing heavy metals from polluted water. Plant assimilation of nutrients and its subsequent harvesting are the other mechanisms for pollutants removal. Low cost and easy maintenance make the aquatic plants attractive to use for remediation of toxic metals from waste water [15]. There has been a growing interest in using metal accumulating roots and rhizomes of aquatic and semi-aquatic vascular plants for the remediation of heavy metals from the municipal waste water and contaminated streams]16]. There are a number of aquatic plants being employed for the removal of heavy metals from waste water [17, 18].

Salvinia molesta (*S. molesta*) is a floating aquatic fern that is used for phytoremediation of heavy metals from waste water. *S. molesta* was used for the treatment of black water effluents in an eco-friendly sewage system [19]. There is a report showing the capability of *S. molesta* in removing Cd, As, Pb, and Cu from the industrial effluents in a constructed wetland system [20]. Ranjitha *et al*. [21] reported that *S. molesta* was able to remove heavy metals like Cr, Cd, Pb, and Cu from industrial effluents. *S. molesta* accumulated metals in roots and fronds through assimilation without any change in the growth regulation process. This plant also contains high percentage of lipids (50-60%), which could be used for the production of biodiesel. The process of waste water treatment involves the take up of waste water from the environment and recycling back into the environment in a suitable form for the betterment of society, ecosystem and biodiversity. The main objectives of this research was to utilize *S. molesta* an aquatic fern for removing heavy metals like Cd, Cu, Ni, Zn, Fe and Pb from the municipal waste water and to use the treated water for irrigation, gardening, cleaning and washing, and to recover the accumulated metals for commercial use. In the present study the concept of rhizofiltration was used to remove toxic metals from waste water using *S. molesta*.

MATERIALS AND METHODS

Municipal waste water samples, plastic trays and bottles, fresh plant specimen, pH paper and ICP-AES instrument.

Sample collection

The municipal waste water was collected from four industrial sites in two different districts of Kerala: a) Kaloor, Kadavanthra Road (near PVS Memorial hospital), Ernakulam b) KSRTC Road, South Chalakudy, Thrissur c) Kadukutty, Kathikudam (Nitta Gelatin), Chalakudy, Thrissur and d) Kokkala, Thrissur, Kerala, India. The sampling was done on 31st August, 4th and 6th September 2015. The samples were collected from 0-1.5 m depth at random from four locations. The color of the waste water was blackish grey and odor was stinky. The maps of sampling sites are shown in Fig. 1. The sampling locations are shown in Fig. 2.

Fig-1: Maps showing different sampling sites: a) Ernakulam, b) Kokkala, c) Chalakudy and d) Nitta Gelatin.

Experimental design

The experiment was carried out in a laboratory set up at room temperature. Two sets of four plastic trays of similar dimensions were used for the experiment. Eight trays were filled with three and half liters of municipal waste water (one replicate of each sampling site). The plants *S. molesta* were collected from the fresh water pond and were cleansed thoroughly. The plants *S. molesta* (31 Nos.) of similar size and weight were introduced into each tray containing municipal waste water. The first set of trays containing waste water was treated for five days and the other set of trays for ten days. The experimental design for waste water treatment is shown in Fig. 3. The waste water sample without plant specimen (Fig. 4) and fresh water with plant specimen were used as controls (Fig. 3). The plants remained alive without decay throughout the experiment. There was no changes in the growth regulation processes. The pH of the samples was recorded before introducing the plants and throughout the experiment. The concentration of heavy metals Cd, Cu, Fe, Ni, Pb and Zn was detected by ICP-AES (STIC, CUSAT, Cochin, Kerala, India).

ICP-AES analysis of municipal waste water

Make of Thermo Electron IRIS INTREPID II XSP DUO, flexible axial and radial view instrument with high concentration capabilities; spectral range: 165 to >1000 nm, resolution: 0.005 nm at 200 nm, detection limit: ppb level, CID detector, sample requirement: 5-10 ml was used for the detection of heavy metals such as Cd, Cu, Fe, Ni, Pb and Zn in the non treated and treated municipal waste water.

Fig-2: Municipal waste water sampling locations: a) & b) Ernakulam, c) & d) Kokkala and e) & f) Chalakudy.

Fig-3: Experimental design for municipal waste water treatment using *S. molesta*: a) & e) Ernakulam, b) & f) Kokkala, c) & g) Chalakudy, d) & h) Nitta Gelatin and i) control (fresh water with plant specimen).

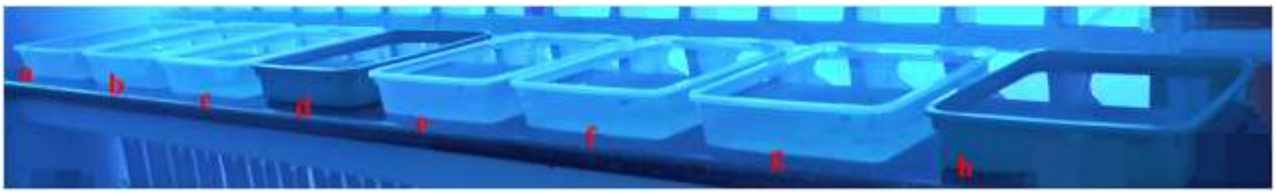

Fig-4: Municipal waste water samples as controls: a) & e) Ernakulam, b) & f) Kokkala, c) & g) Chalakudy and d) & h) Nitta Gelatin.

RESULTS AND DISCUSSION

The present study was involved in the treatment of municipal waste water collected from four different industrial sites in Kerala using an aquatic fern *S. molesta*. The municipal waste water was analyzed before treatment, after five days of treatment and ten days of treatment with *S. molesta* for the estimation of heavy metals such as Cd, Cu, Fe, Ni, Pb and Zn by ICP-AES at Sophisticated Test and Instrumentation Centre (STIC), Cochin University of Science and Technology (CUSAT), Cochin, Kerala, India. The ICP-AES analysis of waste water samples before treatment with *S. molesta* revealed insignificant amount of Cd, Cu, Ni and Pb and significant amount of Fe and Zn in varying concentrations. The concentration of Cu was found to be between 0.005-0.010 ppm. The highest concentration of Cu was seen in sample NG (0.010 ppm) and the lowest in sample KK (0.005 ppm). The amount of Fe was found to be ranging from 0.009 to 0.775 ppm. The highest concentration of Fe was recorded in sample NG (0.775 ppm) and the lowest in samples ER and KK (0.009 ppm). The Ni content of the samples was between 0.006-0.009 ppm. The highest Ni content was recorded in sample NG (0.009 ppm) and the lowest in sample ER (0.006 ppm). The Zn content of the samples ranged from 0.030 to 0.135 ppm. The highest Zn content was seen in sample NG (0.135 ppm) and the lowest in sample KK (0.030 ppm). The ICP-AES analysis of the control is shown in Fig.5. The ICP-AES analysis of the non-treated waste water sample is presented in Fig. 6.

The ICP-AES analysis of waste water samples after five days of treatment with *S. molesta* revealed that the Cu content of the waste water samples was found to be 0.006 ppm (CH), 0.006 ppm (ER), 0.008 ppm (NG). The decrease was by 0.002 ppm in three samples. The Cu content of the sample was below detection limit in sample KK. The concentration of Fe was found to be 0.011 ppm (CH), 0.007 ppm (ER), 0.006 ppm (KK). A huge decrease was seen in Fe content in sample NG (0.038 ppm) after five days of treatment with the plant. The content of Ni ranged between 0.003-0.006 ppm. It was decreased by 0.003 ppm in all samples. The concentration of Zn was estimated to be 0.015 ppm (CH), 0.009 ppm (ER), 0.020 ppm (KK) and 0.121 ppm (NG) respectively. The ICP-AES analysis of waste water sample after five days of treatment is presented in Fig. 7.

The ICP-AES analysis of waste water samples after ten days of treatment with *S. molesta* showed significant amount of reduction in metal concentrations. The results revealed that the Cu and Fe concentrations were below the detection limit in samples CH, ER and KK. Ni content was estimated to be below the detection limit in samples ER and KK. The concentration of Zn was found to be at a reduced level in all the four samples CH, ER, KK and NG. The pH of the waste water samples was also recorded before the experiment and every day of the experiment. The pH of the waste water samples were 7.7 (CH), 7.8 (ER), 7.8 (KK) and 5.5 (NG) before the treatment. The pH of the waste water samples were 7.9 (CH), 8.4 (ER), 8.2 (KK) and 5.9 (NG) after five days of treatment with *S. molesta*. The pH of the waste water samples were 8.8 (CH), 8.9 (ER), 8.8 (KK) and 6.5 (NG) after ten days of treatment with *S. molesta.* The pH of the samples switched from 7.7 to 8.8 (CH), 7.8 to 8.9 (ER), 7.8 to 8.8 (KK) and 5.5 to 6.5 (NG) respectively after ten days of treatment with *S. molesta*. The ICP-AES analysis of waste water sample after ten days of treatment is presented in Fig. 8. A study by Acenas *et al*. ([22] for removing pollutants from black water sewage from a constructed wetland revealed that *S. molesta* was highly efficient for the removal of total suspended solids (30.77%), dissolved oxygen (74.70%) and fecal *coliform* (48.95%) with no effect on pH, chromium, nitrates, sulfates, phosphates and plankton. Microwave-assisted digestion for determination of Pb, Mg, Mn, Cd and Zn in *S. molesta* leaf part by flame atomic absorption spectroscopy (AAS) showed that the content of heavy metals was within the permissible levels except Cd and Pb, suggesting that *S. molesta* can grow healthy with the accumulation of these metals. Further this plant material can also be used for the production of biodiesel [23]. Analysis of heavy metals Cr, Pb, Cu, and Cd from the industrial effluents using *S. molesta* showed that the concentration of heavy metals was less than 10 ppm, suggesting that the plant consumed these heavy metals and still there was no change in the growth regulation processes. This is one of the evidence indicating that *S. molesta* is an accumulator of these toxic metals. The plant also contained 50-60% of lipids [21].

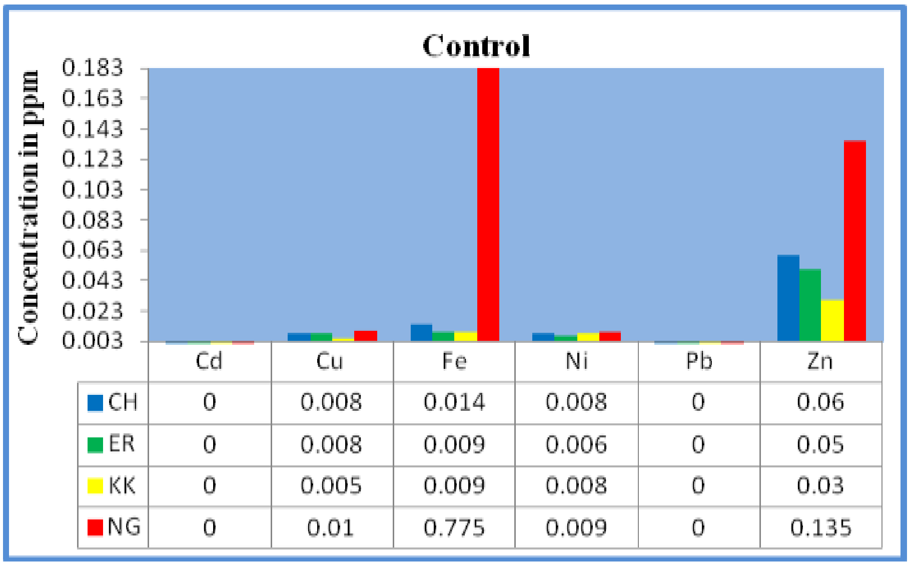

Fig-5: The ICP-AES analysis of heavy metals in the control.

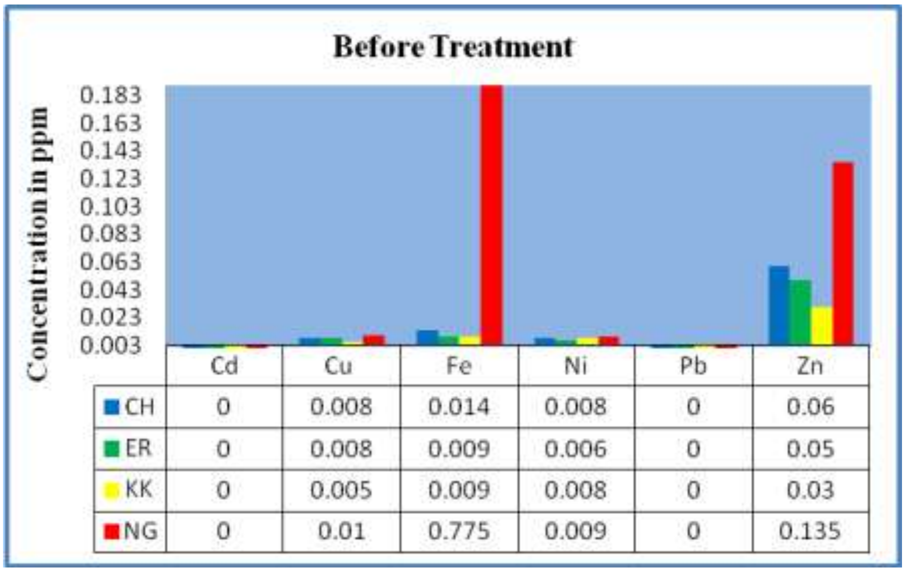

Fig-6: The ICP-AES analysis of heavy metals in the municipal waste water before treatment with *S. molesta* (CH: Chalakudy, ER: Ernakulam, KK: Kokkala and NG: Nitta Gelatin).

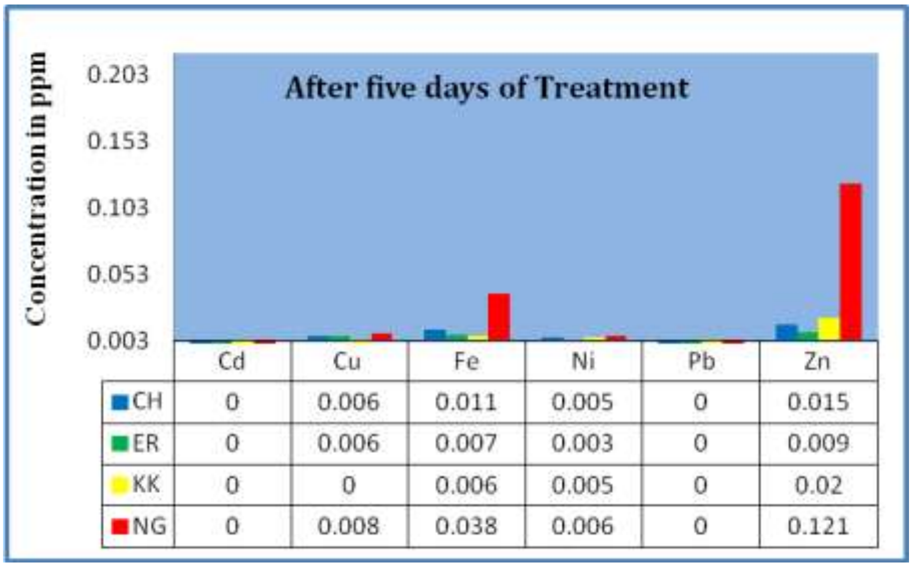

Fig. 7: The ICP-AES analysis of heavy metals in the municipal waste water after five days of treatment with *S. molesta*.

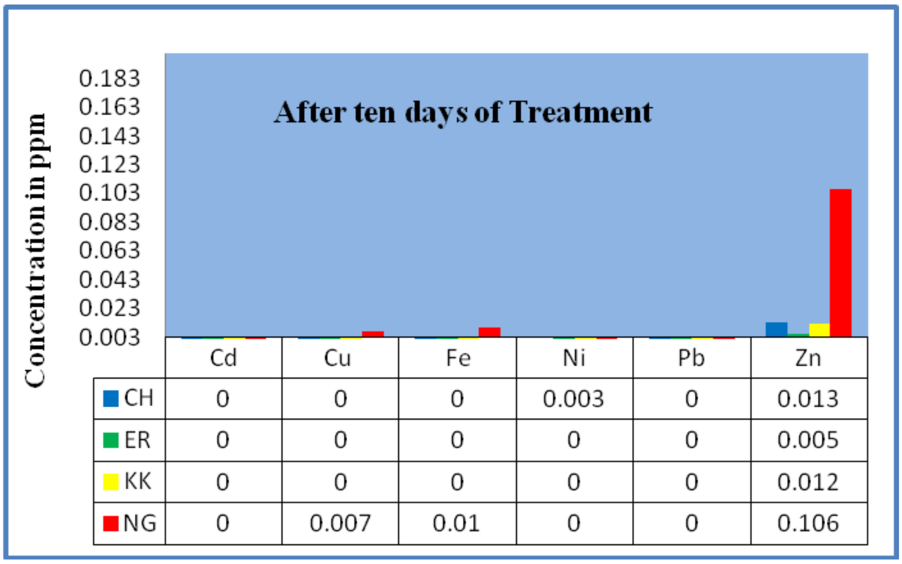

Fig. 8: The ICP-AES analysis of heavy metals in the municipal waste water after ten days of treatment with *S. molesta*.

The determination of nutrient uptake by *S. molesta* from treated Palm Oil Mill Effluent (POME) and its effect towards biomass and biochemical content showed that *S. molesta* achieved 95% phosphate removal from the waste water, lowering concentration to 0.17 mg/l. Nitrate content was found to be 0.50 mg/l. Ammonia concentration exhibited a dynamic fluctuation with minimum value of 2.62 mg/l. Turbidity decreased from 7.56 to 0.94 NTU. The COD removal efficiency was 39%. *S. molesta* also showed increase in the biomass, carbohydrates and protein content showing a superior biochemical composition [24]. An experimental study on the potential of *S. molesta* to reduce the sodium content from the textile effluents has shown that the plant is a suitable candidate for the removal of sodium from the textile waste water [25]. Another investigation has revealed that agricultural residues such as wheat and rice straw together with *S. molesta* biomass could serve as a green technology treatment for the removal of heavy metals from waste water [26].

In the present study, the ICP-AES analysis of the municipal waste water before treatment with *S. molesta* revealed that the metal Fe was present in highest concentration followed by Zn in NG sample. The heavy metals Cu and Ni were present in similar quantity in all samples. The metal ions Cd and Pb were present below the detection limit in all samples. There was a significant decrease in Fe content in NG sample and moderate decrease in Zn content in all samples after five days of treatment. The concentration of heavy metals decreased after five and ten days of treatment with *S. molesta*, revealing that the plant was capable of absorbing and accumulating heavy metal contaminants from the waste water through the process of rhizofiltration. This study explains that *S. molesta* is a hyperaccumulator of Fe ion in an acidic medium (pH of sample NG was 5.5 to 6.5) and it can also remove Zn moderately in alkaline medium (pH:7.7 to 8.8), but requires a longer period of treatment with the plants. The ICP-AES analysis of waste water samples treated for ten days revealed a greater aspect of phytoremediation activity of *S. molesta*. The contents of Cd and Pb were found to be below the detection limit (0.01 and 0.05 ppm) in all the four samples before treatment, after five and ten days of treatment with *S. molesta*. This also indicated that the waste water samples collected from four locations in two districts of Kerala were found to be low in Cd and Pb content when compared to the other elements.

Phytoremediation is one of the innovative approaches to cleanse the polluted environment, such as soil, water and air. The absorption of the contaminants is mainly through the root system of the plants in which the principal mechanism of detoxifying activity is present. The root system renders a larger surface area for the absorption and accumulation of water, essential and non essential nutrients [27], hence the process of rhizofiltration/phytofiltration was used in this study. Plants take time to acclimatize to a newer environment. When the plants were introduced into the waste water samples randomly from the fresh water environment where they were already growing, it must have been a stressing condition for them and took sufficient time to adapt to the new environment. That could be one of the reasons for taking longer period of treatment for removing the metal contaminants in an efficient manner. There was no change in the growth regulation processes of the plants suggesting that the plants could have utilized part of these heavy metals as nutrients for its growth and development. The treated plant material can be safely disposed after the phytoremediation process by burning it and the accumulated metals can be recycled for the commercial purpose. The present investigation is in agreement with the various phytoremediation studies done in *S. molesta* confirming that this plant is a good accumulator of heavy metals and could be used for phytoremediation of waste water.

CONCLUSION

Phytoremediation is a conventional and environment friendly strategy to detoxify the poisonous substances present in the natural environment. Exploring deep into the understanding of the mechanism of it would definitely increase our knowledge level, thus enabling us to choose the appropriate process of phytoremediation and the suitable plant species for the removal of heavy metals in sewage water. Phytoremediation has shown promising results in cleaning up the environment of various pollutants, though it requires more research attempts. The treated water can be used for common purposes like irrigation; gardening, cleaning and washing the corridors in household chores and industries. The present study revealed that *S. molesta* was capable of removing the heavy metal contaminants especially Fe from acidic medium and Zn from alkaline medium, suggesting that this plant could be used as a bioindicator for the determination of heavy metal pollution in waste water. Further research is needed to discover the actual mechanism employed by this plant to scavenge the metal contaminants.

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