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I'm grateful for the opportunity to share my knowledge with you. Please let me know if you have any questions or if there's anything else I can assist you with.
especially with regard to both essential and contaminating metals, and relating it to oxidative stress are lacking. In view of the foregoing, the present study was undertaken to assess the levels of both, essential and toxic metals, at each level of the food chain, from soil, surface and ground water used for irrigation, tap water used for drinking, various types of food crops, metal intakes based on balanced diets, their final concentrations in the blood of residents of selected areas and its impact on their biochemistry. The study was undertaken to compare an agricultural area to represent least pollution, an industrial area which may be comparatively more polluted than agricultural area and a coal mining area to represent a highly contaminated region, in the geographical belt around Allahabad District in Northern India, in order to assess the movement of selected heavy metals through the food chain.

The study was undertaken to assess essential and contaminating minerals in surface, ground and tap water, in soil associated with roots of selected food crops, in different parts of food crops such as roots and leaves of spinach, roots, leaves and tubers of potato, cereals rice, wheat and maize, transfer factors from soil to the edible parts of these food crops, daily intake of metals from balanced diets and health risk index from intakes of these metals. Concentrations and interactions of essential and contaminating metals in blood of control and coal-mining exposed men were also measured, and relationships among blood levels of metals and oxidative stress (OS) markers in these groups were studied. The contaminating metals selected included lead, cadmium, and chromium, and essential metals, copper, zinc, iron, copper and cobalt.

The study areas selected were i) Agricultural area: (AA) comprising of agricultural parts of Jhunsi, Allahabad, ii) Industrial area (IA) comprising industrial areas of Naini, Allahabad, and iii) Coal mining area (CMA) comprising the Singrauli coal mines. For studies on blood concentrations of metals, residents of AA and IA were clubbed to represent Controls (C) not exposed to mining activities, while residents of coal mining area were designated Coal Mining Exposed (CME). Allahabad region (AA and IA in our study) is known to be the least industrialized and least polluted cities in east Uttar Pradesh while Singrauli coal mines are designated as the country’s ninth most critically polluted area by the Ministry of Environment, and are the coal mines closest to Allahabad.
Sampling of soil, water, food crops and blood was conducted in the months of January and February, 2011, at all the selected locations. Soil samples included soil associated to roots of spinach (S1) and potato (S2), cereals (S3), rice, wheat and maize, and reference soil (S4) where no cultivation had been taken up. Food crops included spinach (*Spinacia oleracea*), potato (*Solanum tuberosum*), rice (*Oryza sativa*), wheat (*Triticum aestivum*), and maize (*Zea mays*). Three sources of water samples, surface water (SW), ground water (GW), and tap water (TW) were also collected.

The physico-chemical properties of soil comprising of pH, electrical conductivity, moisture content, water holding capacity (WHC), bulk density, organic matter, were analyzed in all soil samples from AA, IA and CMA. Physico-chemical properties of water, comprising of pH, electrical conductivity, total hardness, total dissolved solids (TDS), turbidity, temperature, dissolved oxygen (DO), in surface, ground and tap water were analyzed following the standard methods described in American Public Health Association. The moisture contents of all soil and crops studied were computed by drying them in an oven till constant weight. Soil and plant samples were digested with tri-acid mixture (HNO₃, H₂SO₄, and HClO₄) at 80°C. Water samples were digested with concentrated HNO₃. Concentrations of Pb, Cd, Cr, Zn, Fe, Cu, and Co in the filtrate of digested soil, edible plant samples, water samples and blood were estimated by using an atomic absorption spectrophotometer (AAS). Transfer factor (TF), daily intake of metals (DIM) and health risk index (HRI) were computed by appropriate formulae.

As described above, blood samples were collected from 60 Control who had no exposure to mining activities, and from 63 coal mining exposed (CME) residents of Singrauli who had frequent and long term exposure to mining activities. Lead, Cd, Cr, Zn, Fe, Cu and Co in their blood was assessed by atomic absorption spectrophotometer. Their iron status was assessed by iron panel comprising of hemoglobin (Hb) and hematocrit (Hct), plasma ferritin, plasma iron (PI) and Total Iron Binding Capacity (TIBC) and percent saturation of transferrin was calculated as the ratio of serum iron and total iron binding capacity. Unsaturated Iron Binding Capacity (UIBC) was also calculated. Oxidative stress was assessed by the erythrocytic metallozyme superoxide dismutase (SOD) , which removes the
superoxide oxygen radical by conversion to hydrogen peroxide (H₂O₂) and plasma glutathione peroxidase (GPx) which reduces lipid hydroperoxides to their corresponding alcohols and reduces free hydrogen peroxide to water. Ferric Reducing Ability of Plasma (FRAP assay) was used to assess the overall oxidative stress levels in plasma. Malonyldialdehyde (MDA) formation which is an index of lipid peroxidation resulting from free radical mediated attack on cell membranes and lipoproteins and Uric acid were estimated in plasma. These parameters form the first line of defence against reactive oxygen species in erythrocytes and in plasma. The erythrocytic membrane is rich in polyunsaturated fatty acids (PUFA), and has an effective mechanism to prevent and neutralize oxidative stress induced damage by the presence of the antioxidant enzymes.

In all water samples, namely surface water, ground water and tap water, temperature, pH turbidity, total dissolved solids, conductivity and total hardness measured as CaCO₃ were highest in CMA followed by IA and were least in AA. Dissolved oxygen was lowest in CMA, followed by IA and was least in AA indicating the highest pollution in the mining area, followed by industrial area. The overall pattern indicated that Pb, Cd, and Cr were lower in tap water than surface water, and groundwater at all three locations. Both surface water and ground water have high amounts of Fe at all locations, followed by Cr. Location affected all samples of water; all heavy metals are higher in IA than AA, and highest in CMA. This pattern was as expected because pollution is likely to be highest in CMA, followed by IA. Lead levels were within safe limits for irrigation purposes. Cadmium and Co levels exceeded safe limits in all types of water at all locations. Cadmium correlated maximally to location, being highest in CMA, followed by Pb. Chromium also exceeded safe limits, except for tap water in AA and IA. Zinc, Fe and Cu were within safe limits for all types of water at all locations.

Soil is a regulator of water quality so the assessment of physico-chemical properties and metal concentration was assessed in soil sample associated with food crops. The overall pattern of physico-chemical properties of soil adhering to various food crops, spinach, potato and cereals at AA, IA and CMA indicate higher electrical conductivity (EC), lower Water Holding Capacity (WHC), lower moisture content and higher bulk
density at coal mining area, followed by industrial area and finally by agricultural area, indicating that AA soil is best for agricultural purposes. All metals studied, except Cd, were within safe limits. Like in water, Cd was higher than this prescribed lower limit at all three locations in soils adhering to spinach and cereals, while in the case of soil adhering to potato; it was higher in CMA only. Iron and Co were higher in soils of CMA. Iron was highest in soil adhering to spinach, followed by cereals while Co was highest in soil adhering to potato. The overall pattern, with minor exceptions, for both, toxic metals Pb, Cd, Cr and for essential metals Zn, Fe and Co, in soil adhering to spinach, potato and cereals were found to be highest in coal mining area, followed by industrial area and least in the agricultural area. Copper seemed to be the sole exception, the levels of which did not seem to depend on location. In order to assess the importance of these findings to human health, transfer of these metals into plants, especially those consumed as food was assessed.

After soil and water, plants form the next link in the food chain. All metals in all food crops were higher in coal mining areas as compared to industrial areas as well as agricultural areas. The overall pattern of metal uptake by food crops from soil indicated that more metals accumulated in vegetables as compared to cereals. Toxic metals Pb, Cd and Cr were highest in CMA and depended on location, while concentrations of essential metals Zn, Fe, Cu and Co in food crops were less consistent. Mean concentration of all the heavy metals were lower in the cereals (wheat, rice, and maize) as compared with the vegetable crops, spinach and potato. Lead, which had not exceeded safe limits in soil and water, was found to exceed safe limits in all food crops at all locations. Safe limits for Cd and Cr was also exceeded in spinach and potato, especially in those grown in coal mining areas. Along with toxic metals, the essential metals were also higher in crop, especially vegetables, grown in coal-mining areas. The metals found in soil, water and food crops are actually available to humans was assessed by parameters like transfer factor (TF), daily intake of metals (DIM) and whether they are causing risk or not was assessed by health risk index (HRI).

Among the food crops studied spinach has the highest transfer factors for all elements with the possible exception of Cr, and depended to some extent on location, being higher for CMA. So if it is grown in contaminated soils it will have toxic elements such as Pb and Cd, while if the soil is rich in essential elements such as Fe and Zn, it
will be rich in these. This was followed by potato and cereals had the lowest transfer factors, which depended on location, being highest for CMA, followed by IA and AA. Rice/wheat contributed maximally to the intake of all elements except Co, which came from drinking water. This is because they were the largest food item in the Indian diet as recommended by ICMR. Most metals had a higher transfer factor in vegetables but contributed less to DIM because of considerably lower recommended intakes.

The HRI for all metals for various crops and drinking water was compared, and among all the metals, Pb and Cd were found to have the highest HRI for vegetables. Transfer of toxic metals Pb and Cd was higher in vegetables; the HRI was higher due to cereals. Therefore, Pb and Cd posed significant health risks for CMA residents, followed by IA and AA in that order, mainly attributable to cereals. Drinking water, however, did not contribute significantly to health risk for any metal studied. Lead and Cd posed significant health risks for CMA residents, followed by IA and AA. Dietary intake of essential metal Fe was also more in the polluted areas of coal mines. This led to investigate the relationships among toxic and essential metals in food with their levels in the blood of residents of these areas.

Significantly higher levels of Pb, Cd, Fe, Cu and Co were found in whole blood of coal mining exposed (CME) as compared to Control respondents, but blood concentrations of Cr and Zn were not dependent on location of residents. Blood Ca was slightly but statistically significantly lower in CME residents, but was within the physiological range. Lead correlated positively with Cd, Cr and Zn, and negatively with Fe, Ca and Cu in CME but not in Controls. Negative correlation was found in Controls but not in CME only in the case of Pb and Co. Blood Cd levels showed negative correlation with Fe and Ca in CME residents as compared to Controls. Iron correlated negatively with Zn, and positively with Co in blood of respondents of CME group but not in blood of Controls. Weak negative correlation of Cu with Zn and Cd, non significant correlations between Zn and Cu levels; Cd and Zn levels was found.

Further, the impact of metals in blood on nutritional status as indexed by hemoglobin, hematocrit and selected markers of oxidative stress were assessed in CME respondents and compared with Controls not exposed to coal mining. It was found that CME respondents had a better Fe status than those not exposed to mining
activities. Coal mining exposed respondents had higher level of Hb, PI and ferritin which was attributed to the fact that Fe in coal is bioavailable and might find way to blood via food chain. Higher MDA, uric acid, and lower SOD and GPx was reported while the overall antioxidant capacity as indexed by FRAP was unchanged. Higher levels of Pb and Cd in their blood were associated with lower BMI, SOD and GPx, and higher MDA. It was indicated that people living in the vicinity of the mining area had higher blood levels of toxic metals Pb and Cd, which correlated with some oxidative stress indices, but not with overall total antioxidant activity, and these effects increased as exposure duration increased. The overall pattern indicated higher oxidative stress in coal mining exposed respondents, but their total antioxidant capacity was not different, indicating that some homeostatic mechanisms are operative.

Among the metals studied in soil, water, food crops and blood in-vivo, Cd and Pb were the two most toxic metals and prominent agents of risk. Hence these were selected for conducting in-vitro experiments on blood samples from Control and coal mining exposed respondents. RBC from blood of respondents of both groups were incubated with salts of Pb and Cd i.e. Pb(NO₃)₂ and CdCl₂. Malondialdehyde (MDA) and superoxide dismutase (SOD) was assessed in erythrocytes incubated with varying doses of Pb(NO₃)₂ (0, 0.1, 0.5, 1.0 and 2.0 µM) and CdCl₂ (0.05, 0.5, 1.0 and 2.0 µM) to cover the permissible and toxic levels in blood. It was found that the initial concentration of MDA was more, and of SOD was less in blood of CME residents than Controls, and this difference continued as concentrations of Pb(NO₃)₂ and CdCl₂ increased. Control and CME showed a large increase in MDA at the highest concentration (2 µM) of both Pb(NO₃)₂ and CdCl₂ and the difference in MDA between Control and CME narrowed down. SOD increased in Controls in response to 0.1µM Pb(NO₃)₂ but declined for all other concentrations of Pb(NO₃)₂, and remained lower for Control than CME. This increased SOD was not observed on incubation with CdCl₂, and both Control and CME respondents showed a Cd induced significant dose related SOD reduction at each concentration.

The overall content of all metals, except Cu, in water, soils, and crops followed the pattern CMA>IA>AA. Transfer factors followed the sequence spinach>potato>cereals. Quantitatively, however, cereals contribute maximally to
balanced diet, so DIM and HRI were higher from cereals than vegetables. Even though spinach had the highest TFs, cereals contributed maximally to HRI. CMA had the highest metal contents so locally grown cereals contributed significantly to intake of both toxic and essential metals.

From this study, insight was gained regarding accumulation and potential effects on health of toxic and essential heavy metals through the food chain from soil, water, vegetables and cereals in various areas such as an agricultural area (AA), industrial area (IA), and coal mining area (CMA). In soil, all metals except Cu were highest in CMA, but levels in IA and AA were similar, except in the case of Pb, which was higher in IA than in AA. In all samples of water, all heavy metals were higher in IA than AA, and highest in CMA. In all food crops in all locations, Pb significantly exceeded both World Health Organization and Indian norms, even though it did not exceed safe limits in soil and water. Spinach and potato showed excessive Cd and Cr also, which was more prominent in CMA.

Lead and Cd exceeded the permissible limits given by World Health Organization in all vegetables and cereals studied, except maize, at all the three locations. The overall pattern, with minor exceptions, for both, toxic metals Pb and Cd and for essential metals Cr, Zn, Fe, and Co, in all parameters studied including soil adhering to spinach, potato, and cereals, irrigation, ground and tap water, foods crops spinach, potato, rice, wheat, and maize, transfer factors for soil to plant, daily intake of metal from each food and from total diet, and health risk indices for each food item and total from the diet were found to be highest in the coal mining area, followed by industrial area and least in the agricultural area. Copper seemed to be the sole exception, the levels of which did not seem to depend on location.

Spinach had the highest transfer factors, so if it is grown on soils contaminated with toxic elements such as Pb and Cd, it accumulates these, and if grown in soils rich in essential minerals, especially Fe and Zn, it becomes rich in these. This was followed by potato and cereals had the lowest transfer factors, which depended on location, being highest for CMA, followed by IA and AA.
However, cereals form the quantitatively largest staple food item in the Indian diet; hence these contribute more to the daily intake of all metals than spinach and potato, which form a smaller proportion of the diet. Thus, even though transfer of toxic metals Pb and Cd was higher in vegetables, health risk index was higher due to cereals. Therefore, Pb and Cd posed significant health risks for CMA residents, followed by IA and AA in that order, mainly attributable to cereals, while drinking water did not contribute significantly to intake of any metal studied, except Co. It is noteworthy that dietary intake of essential metal Fe was also more in the polluted areas of coal mines. Iron deficiency is one of the most widespread nutrition problems worldwide, so this observation needs to be evaluated in terms of Fe status indicators of residents in these areas.

These patterns are also visible in blood of the coal mining exposed group, who had high levels of blood Pb, Cd and Fe. The bioavailability of iron was indicated, but it was accompanied by increase in lipid peroxidation, and some modifications in antioxidant enzymes SOD, GPx, and total antioxidant capacity, FRAP and uric acid. The overall pattern was indicative of increased oxidative stress which showed relationships with blood lead and cadmium.

This study contributes to present knowledge regarding the risks associated with regard to high levels of both, essential and contaminating metals in Singrauli coal mines, which could be seen throughout the food chain starting from soil to water to food to blood of residents, and indicated increased oxidative stress, but also better iron status. Studies are lacking which deal with the complete food chain in this geographical belt. Hence, it is submitted that the present study has attempted to fill the gap in knowledge regarding interrelationships among metals at each step of the food chain and their relationship to oxidative stress in the respondents of coal mining area as compared to non-mining control respondents. This study becomes important in the light of the fact that the metals in mining areas are finding out their way in blood of miners and causing oxidative stress, highlighting the need to develop strategies and introduce measures to ensure health of mine workers.