Published in final edited form as:

Metallomics. 2011 November ; 3(11): 1153–1162. doi:10.1039/c1mt00063b.

Mechanisms of nickel toxicity in microorganisms

Lee Macomber^a and Robert P. Hausinger^{a,b}

Robert P. Hausinger: hausinge@msu.edu

^aDepartment of Microbiology and Molecular Genetics, Michigan State University, East Lansing, Michigan 48824-4320, USA

^bDepartment of Biochemistry and Molecular Biology, Michigan State University, East Lansing, Michigan 48824-1319, USA

Summary

Nickel has long been known to be an important human toxicant, including having the ability to form carcinomas, but until recently nickel was believed to be an issue only to microorganisms living in nickel-rich serpentine soils or areas contaminated by industrial pollution. This assumption was overturned by the discovery of a nickel defense system (RcnR/RcnA) found in microorganisms that live in a wide range of environmental niches, suggesting that nickel homeostasis is a general biological concern. To date, the mechanisms of nickel toxicity in microorganisms and higher eukaryotes are poorly understood. In this review, we summarize nickel homeostasis processes used by microorganisms and highlight *in vivo* and *in vitro* effects of exposure to elevated concentrations of nickel. On the basis of this evidence we propose four mechanisms of nickel toxicity: 1) nickel replaces the essential metal of metalloproteins, 2) nickel binds to catalytic residues of non-metalloenzymes; 3) nickel binds outside the catalytic site of an enzyme to inhibit allosterically, and 4) nickel indirectly causes oxidative stress.

1.0 Introduction

Nickel concentrations vary widely in different environmental niches, with low nanomolar [i.e., ~0.1 µg/g (ppm)] to low micromolar [i.e., ~0.1 ng/g (ppb)] levels in many aquatic systems, 10–40 ppm in most soils, and several 100 ppm in serpentine soils.¹ In addition, nickel is a common industrial pollutant with concentrations in wastewater around industrial parks sometimes reaching the low millimolar range.² This metal is typically found in the Ni(0) or Ni(II) state due to the stability of these species in water.³ Nickel toxicity to humans has received intensive attention due to nickel's links to cancer.^{4–7} Similarly, nickel toxicity to plants is well documented.⁸ Until recently, nickel toxicosis in microorganisms was believed to be a problem limited to cells exposed to industrial pollution or found in naturally-occurring nickel-contaminated soils.⁹ This assumption was overturned, however, when a nickel defense system (RcnA) was characterized in the mesophile *Escherichia coli*.¹⁰ Furthermore, putative homologues of RcnA were detected in archaea and throughout bacteria.¹⁰ This discovery suggests that excessive nickel is a routine physiological concern of microorganisms. For example, commensal bacteria in the human gut are exposed to 150 –

900 µg of nickel per day as a constituent of the diet.¹¹ In this review we summarize what is known about Ni(II) homeostasis and toxicity in microorganisms and we discuss potential mechanisms of nickel toxicosis.

2.0 Nickel homeostasis in microorganisms

Nickel is used as a cofactor by several well-characterized microbial enzymes including urease, [NiFe] hydrogenase, Ni-superoxide dismutase, carbon monoxide dehydrogenase, acetyl CoA synthase/decarbonylase, acireductone dioxygenase, and methyl coenzyme M reductase, as well as some forms of glyoxalase I.^{12–15} Additional nickel-dependent enzyme activities are reported, and include glycerol-1-phosphate dehydrogenase from *Bacillus pasteurii*¹⁶ and quercetinase from a *Streptomyces* species.¹⁷ The metalcenter of nickel enzymes typically is coordinated by histidine and/or cysteine residues, with additional contributions from aspartate, glutamate and, in the case of urease, a lysine carbamate. In addition, some proteins bind nickel using the amino terminal amine or backbone amide groups.^{12–15}

In organisms with nickel-containing proteins, mechanisms have evolved to take up the metal. At the same time, nickel efflux or other detoxification systems have evolved to overcome problems of excessive concentrations of this metal. Accompanying these uptake and resistance mechanisms, microorganisms often contain proteins for sensing and responding to nickel. Finally, some microbes appear to employ nickel storage proteins.¹⁵ Together these processes provide mechanisms for nickel homeostasis in microorganisms.^{12, 13, 18–22} Microbes generally concentrate metals from their growth medium,^{23, 24} and this is true also for nickel. For example, *E. coli* cells grown on M9 minimal medium (containing ~1 µM nickel) contained ~30 µM of this metal (unpublished observation). Whereas the total concentration of nickel in a microorganism can be ascertained, this value does not equate to the concentration of the free metal in the cell.

2.1 Nickel metalloregulatory proteins

Several DNA-binding proteins undergo conformational changes upon binding nickel, leading to changes in transcription of genes encoding metal transport and nickel proteins. NikR is the best-studied nickel-dependent regulatory protein.²⁵ Tetrameric NikR from *E. coli* binds four nickel at high-affinity sites,^{26, 27} with additional sites for binding potassium and (with lower affinity) additional nickel,²⁸ and the nickel-bound protein serves as a repressor by binding to specific operator sequences.²⁹ NikR proteins of other organisms exhibit differences in metal binding and regulatory properties.^{30–33} Working in the opposite manner is RcnR, an *E. coli* protein that releases from the *rcnA* promoter DNA in the presence of nickel, thus allowing transcription to take place.³⁴ Studies on the ArsR-SmtB family of metal-sensing transcriptional repressors in *Mycobacterium tuberculosis* discovered two members (NmtR and KmtR) that sense nickel and cobalt.^{35, 36} Some of the actions of these regulatory proteins are highlighted in the following sections.

2.2 Nickel import

According to comparative genomic analysis,³⁷ the most widespread mechanism for high-affinity uptake of nickel in eubacteria and archaea utilizes an ATP-binding cassette (ABC) system, such as NikABCDE, whereas the most common mechanism in eukaryotes (also widely distributed in bacteria) involves members of the nickel/cobalt transporter (NiCoT) family. In addition, nonspecific transporters of nickel are used by some microorganisms to facilitate transfer of the metal across the cytoplasmic membrane. Finally, bacteria with an outer membrane have, in some cases, evolved mechanisms to expedite nickel transport across that barrier. These topics, briefly summarized below, have been reviewed previously^{12, 13, 38, 39} and are illustrated in the lower portion of Figure 1.

NikABCDE was first characterized in *E. coli* and shown to be a part of the ABC transporter subfamily 2.⁴⁰ NikB and NikC are transmembrane proteins that form a nickel pore.⁴⁰ NikD and NikE are proteins that bind to and hydrolyze ATP.⁴⁰ NikA is a periplasmic protein that binds one nickel per protein along with a naturally-occurring organic metallophore; the structure of this nickel chelate remains unknown but is thought to be a tricarboxylate.⁴¹ Nik homologues, sometimes known as Cbi/NikMNQO and often lacking the periplasmic component, have been found in many bacteria. A few examples of other Nik systems include those in *Brucella suis*,⁴² *Vibrio parahemolyticus*,⁴³ *Helicobacter hepaticus*,⁴⁴ *Yersinia sp.*,⁴⁵ and *Staphylococcus aureus*.⁴⁶ The NikABCDE import system is transcriptionally regulated by NikR,²⁵ whose gene is in turn regulated by FNR.⁴⁷

The founding member of the NiCoT family is HoxN, a high-affinity nickel permease found in *Cupriavidus necator* (formerly *Alcaligenes eutrophus*) and required for full urease and hydrogenase activities.^{48–50} This family can be divided into those specific to nickel, those capable of transporting both nickel and cobalt, and those with a preference for cobalt.³⁹ NiCoT proteins generally exhibit a low K_m (10–20 nM) and a low transport capacity for their metal.^{50, 51} NiCoT representatives have been identified in a wide range of bacteria, including *Rhodococcus rhodochrous*,^{52, 53} *Bradyrhizobium japonicum*,⁵⁴ *H. pylori*,⁵⁵ and *Mycobacterium tuberculosis*.⁵⁶ Family members additionally are prevalent in eukaryotic microorganisms,³⁹ as first demonstrated for *Schizosaccharomyces pombe*.⁵⁷ The energy source required to import nickel by NiCoT proteins remains unknown.¹³

Nonspecific nickel import into the cytoplasm also occurs. For example, nickel can enter *E. coli* through the magnesium importer CorA.⁵⁸ Deletion of *E. coli* genes encoding both Nik and CorA still left the strains nickel sensitive,⁴⁰ therefore this metal must be capable of entering the cytoplasm through other channels. Entry of nickel through CorA and other adventitious transporters likely serves as the route of nickel entry under high environmental nickel concentrations.

Nickel must pass an additional barrier in eubacteria containing an outer membrane. The metal initially was thought to passively cross through porins; however, *H. pylori* urease activity was shown to be inhibited by knocking out the TonB-dependent transporters (TBDTs) FecA3 and FrpB4.^{59, 60} Putative TBDTs of nickel have since been found in several other organisms.^{61, 62}

In summary, several distinct types of mechanisms are used to import nickel into microbial cells. This metal has a beneficial role when incorporated into nickel-dependent enzymes, but it can be toxic when present in excessive amounts.

2.3 Nickel efflux

Microbes are subject to their environmental conditions and, as such, they are exquisitely vulnerable to changes in metal concentrations. Compounding this issue is their tendency to concentrate metals.^{23, 24} For these reasons microorganisms have evolved ways to protect themselves from metal overload. A common microbial response to elevated concentrations of a toxic metal is to synthesize a specific efflux system, thus reducing the internal concentration of that metal species.⁶³ Nickel efflux pumps are best characterized in organisms exhibiting hyper-resistance to this metal, typically isolated from nickel-rich, polluted or naturally-occurring soils. Two examples of nickel-resistant organisms obtained from metal-contaminated industrial sites are *Cupriavidus* (formerly *Wautersia*, *Ralstonia*, or *Alcaligenes) metallidurans* and *Alcaligenes* (or *Achromobacter) xylosoxidans*.^{64, 65} Nickel efflux pumps also are present in non-extremophiles, as exemplified by *E. coli*¹⁰ and *H. pylori*.⁶⁶ Below, we summarize features of nickel efflux (see the upper half of Fig. 1) in these microorganisms as representatives of many other species.

Work in *C. metallidurans*, *A. xylosoxidans*, and *H. pylori* identified several nickel-resistance determinants. The plasmid-borne gene clusters *cnrCBA* (cobalt and nickel resistance)⁶⁷ and *nccCBA* (nickel-cobalt-cadmium resistance)⁶⁸ and the chromosomal *cznCBA* (cadmium, zinc, and nickel resistance)⁶⁶ encode members of the resistance nodulation division (RND) family containing inner membrane, periplasm, and outer membrane components and driven by the proton gradient. These proteins are believed to pump periplasmic metals out across the outer membrane, and thus are not found in bacteria lacking outer membranes.⁶⁹ Another plasmid-encoded protein, NreB, is part of the major facilitator superfamily (MFS) and likely utilizes the proton motive force to pump nickel out of the cytoplasm.⁷⁰ Expression of *nreB* in *C. metallidurans* or *E. coli* increases cellular resistance to nickel challenge. Homologues of *nreB* exist in several other bacteria.^{63, 71} Finally, *C. metallidurans* also contains chromosomally-encoded DmeF efflux pump that confers resistance to iron, zinc, cobalt, cadmium, and nickel.⁷² This gene is constitutively expressed and is not induced by any of the listed metals, but additional findings led to the conclusion that DmeF plays a central role in cobalt homeostasis.⁷²

The RcnA efflux pump was first identified in *E. coli* and shown to pump nickel and cobalt out of the cytoplasm.¹⁰ Putative homologues exist in alpha, beta, and gamma proteobacteria, cyanobacteria, and archaea.¹⁰ Expression of *rcnA* is regulated by the nickel/cobalt responsive repressor, RcnR. As expected, the relative nickel-binding affinities of RcnR ($K_{app} < 26$ nM) and NikR ($K_d = 1$ pM)¹⁹ determine the differential transport activities of RcnA and NikABCDE, and thus poise the internal nickel concentration of the cell.¹⁹ The discovery of the chromosomally-encoded RcnA in the mesophile *E. coli* suggests that environmental nickel is a concern to many microorganisms under typical conditions.

Additional representative exporters of nickel include a putative metal efflux P₁ type ATPase (*nmtA*), repressed by NmtR, and a putative metal efflux pump (*cdf*) of the cation-diffusion-facilitator family, repressed by KmtR.^{35, 36}

2.4 Other nickel resistance mechanisms

Although nickel efflux is widely used by cells to protect against elevated concentrations of this metal, several other mechanisms are utilized by selected microorganisms. *E. coli* exhibits a chemotactic response to environmental nickel concentrations. Nickel acts as a chemotactic repellent;^{73–75} and this response is dependent on the methyl-accepting chemotaxis protein Tar and is independent of NikA.⁷⁵ Nickel-resistant strains of *Saccharomyces cerevisiae* have long been known to sequester the metal in a vacuolar fraction along with high concentrations of the amino acid histidine.^{76, 77} Genetic studies revealed that histidine auxotrophs exhibit enhanced sensitivity to nickel, along with several other metals, and this work showed that strains containing a defective vacuolar proton ATPase (thus unable to acidify the vacuole) display greater nickel toxicity.⁷⁸ In another yeast study, genetic knockouts of iron import sensitized the cells to nickel.⁷⁹ More generally, genome-wide analyses of *S. cerevisiae* identified 149 genes that, when deleted, led to increased nickel sensitivity consistent with having a role in nickel resistance.⁸⁰ Some of these genes relate to proton transporting ATPases, but cation transporters, siderophore-iron transporters, diphthamide biosynthesis, and other processes are included. Sulfate-reducing bacteria produce copious amounts of sulfide that can abrogate the toxicity of the metal. For example, a nickel-resistant strain of *Desulfotomaculum* cultured with high concentrations of this metal produces a dark-brown, soluble, nickel-sulfide product as a means to reduce the concentration of the free ion.⁸¹ *Pseudomonas putida* S4 accumulates nickel in its periplasm, possibly in complex with an 18 kDa protein, as a mechanism of resistance to this metal.⁸² By contrast, a strain of *Pseudomonas aeruginosa* accumulates intracellular nickel which, according to energy-dispersive X-ray analysis, is in the form of metallic nickel.⁸³ The reduction of Ni(II) to elemental nickel also is observed in *Thiocapsa roseopersicina* when grown on hydrogen gas, and this reduction is linked to greater nickel tolerance.⁸⁴

The distribution of nickel defense systems in hyper-resistant as well as more common microorganisms including *E. coli* suggests that excessive nickel is a widespread physiological concern. This leads to the question: What do the nickel defense systems protect? The following section focuses on potential cellular targets of nickel toxicity in microorganisms.

3.0 Nickel toxicity

As illustrated by the plethora of review articles on nickel toxicity toward humans, other animals, and plants (e.g., ^{4–8}), much is known about how this metal harms the cells of higher eukaryotes. Much of the early literature focused on the toxic and carcinogenic effects of particulate nickel compounds on ore miners, with additional studies relating to the toxicity of nickel carbonyl (used in refining) and immunological effects in nickel dermatitis. The mechanisms of nickel damage to humans had long emphasized oxidative reactions involving lipids, proteins, and DNA, but nickel also was known to bind to various biomolecules and

change their properties.⁴⁻⁷ Only recently has nickel been shown to have profound effects on DNA repair and DNA or histone methylation, in the latter case resulting in epigenetic effects.⁸⁵⁻⁸⁷ In contrast to this wealth of information, the mechanisms of nickel toxicity in microorganisms have been understudied, despite the widely demonstrated toxic effect of this metal.⁹

Several strategies can be used to establish the cellular targets of nickel toxicosis. At the organismal-level, clues may be gained by examining the nickel sensitivity for cells grown under varied conditions (e.g., use of different nutrients, aerobic vs. anaerobic conditions, treatment with other stresses, etc.), by transcriptomic or proteomic comparisons to identify genes or proteins synthesized during nickel stress, and by mutational analysis to identify genes that, when mutated, result in enhanced resistance to the metal. An example of the proteomic approach is provided by analysis of nickel-stressed *Pseudomonas putida*.⁸⁸ A range of proteins with altered expression was identified including several up-regulated proteins involved in antioxidant defense. An extensive deletion mutant screen of *S. cerevisiae* nicely demonstrates how that approach can be exploited.⁸⁰ Enhanced resistance was associated with deletions of 119 genes, including those causing derepression of glucose-repressed genes. In addition to the above organismal approaches, more targeted studies to define the sites of nickel toxicity focus on direct biochemical assays in the presence and absence of the metal ion. For example, the effects of nickel on DNA or lipid modifications have been examined. In addition, enzyme activities can be measured using intact cells, cell-free extracts, partially enriched samples, or purified enzymes with the results used to identify potential targets of nickel inhibition. Several examples of nickel-inhibited enzymes are described below. It is important to emphasize, however, that nickel inhibition of an enzyme activity measured *in vitro* must be verified for its *in vivo* relevance—a situation that is seldom realized.

Observations from nickel-toxified cells and measurements of nickel's *in vitro* effects on biomolecules can be synthesized into four general hypotheses: 1) nickel replaces the essential metal of metalloproteins, 2) nickel binds to catalytic residues of non-metal enzymes, 3) nickel binds outside the catalytic site of an enzyme to inhibit allosterically, and 4) nickel leads to oxidative stress that can affect proteins, DNA, or lipids. These generalized toxicity mechanisms are examined in greater detail below with examples of binding at secondary sites folded in with the inhibition of metalloenzymes and non-metal enzymes.

3.1 Nickel toxicity involving metalloproteins

Metals participate in a variety of essential biological functions, including metalloregulation, structural stabilization, electron transfer, substrate/cofactor coordination, and catalysis. The atomic sizes, ligand affinities, preferred coordination geometries, redox states, and available metal concentrations dictate, in part, the identity of the bound metals, with accessory proteins also controlling the metal speciation in selected enzymes.⁸⁹ Although cells attempt to maintain precise intracellular metal concentrations, this homeostasis can fail when excess nickel is present and lead to adventitious displacement of the cognate metal. Replacement of the normal metal by nickel in a metalloregulatory protein could reasonably interfere with its function. In contrast, substitution of nickel into a structural site is unlikely to exhibit severe

repercussions. Incorporation of this metal into an electron transfer site that normally contains iron or copper likely will inhibit function due to the stable nature of the Ni(II) state. Below, we focus on a few illustrative examples where nickel either substitutes for a catalytic metal ion or binds at a second site on a metalloenzyme to inhibit catalysis (Fig. 2 and Table 1).

3.1.1 Nickel inhibition of iron metalloenzymes—A clear connection exists between nickel toxicity and iron metabolism. In *E. coli*, Fur was found to regulate *rcnA* in an iron-dependent manner.⁹⁰ Also, iron supplementation induced *rcnR* transcription in a Fur-independent manner.⁹⁰ Furthermore, the Fur-regulated gene *yqjH* of *E. coli*, shown to be a ferric reductase that plays a role in iron acquisition, is also regulated by nickel through YqjI.^{91, 92} The authors demonstrated that *yqjH* mutants are sensitive to micromolar levels of nickel under conditions that require significant production of the [FeS]-cluster enzyme 6-phosphogluconate dehydratase for growth. These results suggest that *E. coli* may need increased iron uptake during nickel overload,⁹² a situation that is likely to apply to many more organisms. A requirement for increased iron when cells are stressed with nickel is compatible with the mis-incorporation of nickel into iron metalloenzymes. As illustrated below, many iron-containing enzymes are inhibited by nickel, with some shown to have nickel replacing the active metal.

Many iron- and α -ketoglutarate (α KG)-dependent dioxygenases are inhibited by nickel, likely via direct displacement of the active site iron (Fig. 3). These enzymes possess a mononuclear, nonheme ferrous ion at the active site and couple the oxidative decarboxylation of α KG to the oxidation of their diverse primary substrates.^{93–95} One of the better studied examples is taurine/ α KG dioxygenase (TauD), an *E. coli* enzyme that releases sulfite from aminoethanesulfonate as part of a sulfur assimilation pathway. *In vitro* experiments starting with the iron-loaded enzyme measured an IC_{50} for nickel of 32 μ M, whereas the IC_{50} decreased to 0.7 μ M Ni(II) if iron was added after nickel exposure.⁹⁶ These results imply that taurine/ α KG dioxygenase will be more susceptible to nickel during iron limitation or immediately after synthesis. Notably, nickel was shown to exhibit slow-binding inhibition kinetics with an initial K_i of 166 μ M and a maximum inactivation rate constant of 0.078 s^{-1} ; thus, nickel is not a potent inhibitor of TauD. Another iron/ α KG-dependent dioxygenase is the fungal enzyme thymidine 2'-hydroxylase (pyrimidine-deoxynucleoside 2'-dioxygenase) from *Neurospora crassa* which lost 95% of its *in vitro* ability to hydroxylate pyrimidines upon exposure to 250 μ M Ni(II).⁹⁷ Inhibition of the Fe/ α KG-dependent dioxygenases in microorganisms parallels the findings in mammalian systems where nickel inhibits histone demethylases (JMJD1A, JMJD2, and JMJD2E), oxygen-sensing prolyl 4-hydroxylases (PHD2, EGLN1), and DNA repair nucleotide demethylases (ABH2 and ABH3) by replacement of the catalytic iron atom.^{85–87} Inhibition of these chromatin modification enzymes may explain the nickel-dependent mutagenic and epigenetic phenotypes observed in mammals.^{6, 85, 98} Nearly all members of this enzyme family contain an iron-binding motif (comprised of H-X-Asp/Glu-X_n-H) that binds iron using only three residues and is, thus, likely to compete for nickel. Significantly, structures of several family members, crystallized in the presence of nickel to avoid iron-based redox

chemistry, show the inhibitory metal is bound to the iron-binding motif (e.g., PDB ID codes 2ox0, 2w2i, 2wwu, 2xlm, 2xxz, 3k2o, 3ai6, and 3ms5).

Several other ferrous ion-dependent dioxygenases also are inhibited *in vitro* by nickel, presumably due to the relatively redox-stable inhibitor replacing the active metal. For example, protocatechuate 3,4-dioxygenase from *Rhizobium leguminosarum* (*trifolii*) lost 72% of its activity after exposure to 10 μM Ni(II):EDTA.⁹⁹ This enzyme cleaves the aromatic ring of protocatechuate by adding dioxygen to form β -carboxy-*cis,cis*-muconate, thus playing an important role in aromatic catabolism. In an analogous manner, catechol 1,2-dioxygenase cleaves catechol to *cis,cis*-muconate. When exposed to 10 μM Ni(II):EDTA this enzyme, also from *R. leguminosarum*, similarly lost 85% of its activity.¹⁰⁰

In addition to ferrous ion-dependent dioxygenases, many other iron-dependent enzymes have been shown to be sensitive to nickel. As one example, ATP:cob(I)alamin adenosyltransferase (EutT) from *Salmonella enterica* is thought to be an iron-containing enzyme^{101–103} that catalyzes the adenylation of cobalamin (vitamin B₁₂) to adenosylcobalamin (coenzyme B₁₂) for use in ethanolamine catabolism.^{101–104} Purified EutT lost 50% of its activity upon exposure to 100 μM Ni(II).¹⁰² Of interest, nickel concentrations higher than 100 μM did not decrease EutT activity below 50%¹⁰² implying either that nickel binds to an allosteric site, rather than displacing the catalytic iron, or that EutT:Ni catalyzes the adenylation of cobalamin at a reduced rate compared to the iron-bound species. Atrazine chlorohydrolase (AtzA) from a *Pseudomonas* sp. is another putative iron enzyme that is sensitive to nickel inhibition.¹⁰⁵ This enzyme hydrolytically dechlorinates the herbicide atrazine to form hydroxyatrazine. Exposure of isolated enzyme to 200 μM Ni(II) led to the loss of 60% AtzA activity.¹⁰⁵ The authors determined that Zn(II) and Cu(II) also inhibit the enzyme, in these cases without displacing the active site metal, so it is possible that nickel inhibits this enzyme by binding at an allosteric site.¹⁰⁵

3.1.2 Nickel inhibition of zinc metalloenzymes—Several zinc metallopeptidases have been shown to be susceptible to micromolar levels of nickel. The *Trypanosoma cruzi* metallocarboxypeptidase 1 (TcMCP-1), which cleaves the carboxyl-terminal amino acid from peptides and proteins, is one example. Purified TcMCP-1 lost 54% of its activity upon incubation with 10 μM Ni(II).¹⁰⁶ This enzyme utilizes two histidines (H267 and H271) and a glutamate (E296) to coordinate the active site zinc,¹⁰⁶ and these residues are likely to function in nickel binding. Similarly, aminopeptidases from *Streptomyces septatus* (SSAP) and *Streptomyces griseus* (SGAP) lost 47% and 60% of their activities, respectively, after incubation with 100 μM Ni(II).¹⁰⁷ SSAP and SGAP are members of a metallopeptidase family containing dinuclear zinc sites.^{107–109} One zinc atom is coordinated by a histidine and an aspartate, the other is coordinated by a second histidine and glutamate, and the two zinc atoms are bridged by an aspartate.¹⁰⁸ A tripeptidase from *Pediococcus pentosaceus*, also suggested to contain a dinuclear zinc site, was found to lose 94% of its activity after exposure to 100 μM Ni(II).¹¹⁰ Prenyl protease Rce1p, a putative zinc metallopeptidase from *S. cerevisiae*, also is sensitive to nickel with mid-micromolar levels of nickel (87 μM) leading to 48% inhibition of the enzyme.¹¹¹ The mechanism by which nickel inhibits these enzymes was not determined, but competition between zinc and nickel for enzyme binding is likely.

3.1.3 Nickel inhibition of other metalloenzymes—An exhaustive search likely would uncover a large number of other metalloenzymes that are inhibited *in vitro* by nickel, but in few cases are any mechanistic details available. Three examples are illustrated here. The copper-containing enzyme nitrous oxide reductase from *Rhodobacter sphaeroides*¹¹² is inhibited by nickel. Interestingly, zinc inhibition of the enzyme did not lead to loss of the copper¹¹² which could mean that nickel inhibition also may be independent of copper loss. The second example concerns a case where the enzyme uses metal as the substrate rather than for catalysis. Cobaltochelatase from *Pseudomonas denitrificans* inserts cobalt into hydrogenobyrinic acid a,c-diamine during vitamin B₁₂ biosynthesis, and this activity was shown to be blocked by nickel.¹¹³ The exact mechanism of cobalt insertion has yet to be determined. It is conceivable that nickel competes with cobalt for binding to the enzyme or that nickel binds to and thus sequesters the required thiol. Finally, nickel has variable effects on the magnesium-dependent DNA polymerases.¹¹⁴ For example, nickel substitutes for the usual metal in *E. coli* DNA polymerase I, is a weak inhibitor of the bacteriophage T4 enzyme, and is a more effective inhibitor of the enzyme from bacteriophage T7.

3.2 Nickel toxicity involving non-metal enzymes

Nickel also is known to inhibit a wide selection of enzymes that do not require a metal for catalysis. In general terms, such inhibition could occur by nickel binding to active site residues (Fig. 4), thus blocking catalysis, or, as mentioned above, by the metal binding at a secondary site to influence activity allosterically (Fig. 2). In most cases, the mechanism of inhibition is unknown.

Several enzymes shown to be inhibited by nickel are known to contain catalytic cysteine residues. For example, *N*-carbamoyl D-amino acid amidohydrolase from *Agrobacterium* uses a Cys/Glu/Lys catalytic triad to produce D-amino acids^{115, 116} which are important for the production of β -lactam antibiotics. Wang et al.¹¹⁶ found that the enzyme additionally contains three histidine residues important for enzyme function. This enzyme is sensitive to nickel-dependent inhibition at concentrations as low as 100 nM,¹¹⁷ though its mode of binding the inhibitory metal is unknown. Pyroglutamyl peptidase I, a member of the C15 family of cysteine peptidases, functions to hydrolyze N-terminal L-pyroglutamate residues which may play a role in protein metabolism and defense against antibiotic peptides.^{118, 119} Exposure of the enzyme from *Leishmania major* to 100 μ M Ni(II) causes the loss of 48% of its activity.¹²⁰ Mercury(II) reductase plays a role in reduction of Hg(II) to Hg(0), thus detoxifying the solution by converting the metal ion to a volatile species. The enzyme from *E. coli* (MerA) contains four cysteine residues¹²¹ which might relate to why the protein from *A. chroococcum* is sensitive to 100 μ M Ni(II), causing a loss of 62% activity.¹²² The tRNA-modifying enzymes cytosine-5 methyltransferase and uracil-5 methyltransferase from *E. coli* strain W are sensitive to nickel.¹²³ These enzymes contain catalytic cysteine residues which may explain why activity for both enzymes decreases by 80% upon incubation with 1 μ M Ni(II).^{123–125}

Many other enzymes lacking Cys at their active sites are inhibited by nickel, but the inhibitory mechanisms are generally unknown. Two examples are cited. Arabinose isomerase, an enzyme utilizing catalytic histidine and glutamate residues,¹²⁶ loses 60% of its

activity when incubated with 10 μM Ni(II).¹²⁷ Uricase (urate oxidase) from *Bacillus fastidiosus* exposed to 100 μM Ni(II) loses 81% of its activity;¹²⁸ this enzyme may employ a histidine-threonine-lysine triad for activity.^{129, 130}

3.3 Nickel toxicity via oxidative stress

Oxidative stress has long been discussed as a contributing factor to nickel carcinogenesis in humans.^{4–7} As such, there is much interest in studying the relevance of oxidative stress to nickel toxicity in microorganisms. Many studies have shown that cells subjected to oxidative stress exhibit enhanced DNA damage, protein impairment, and lipid peroxidation along with increased titers of oxidative stress defense systems.^{131, 132} Below, we summarize the types of nickel-dependent oxidative damage noted *in vitro* and describe a few examples where *in vivo* signatures of oxidative damage by nickel are reported.

3.3.1 *In vitro* studies—Nickel is a rather poor generator of oxidative damage when compared to iron or copper;¹³³ nevertheless, this metal can modify many types of biological compounds. For example, nickel can participate in DNA cleavage,^{134, 135} intrastrand cross-links,¹³⁶ deglycosylation,¹³⁷ and base modification.^{136–141} Furthermore, nickel can lead to protein cleavage¹⁴² or oxidize side chains to produce Tyr-Tyr cross-linkings.¹⁴³ In addition, this metal can stimulate lipid oxidation in the presence of thiols¹⁴⁴ or when combined with iron.¹⁴⁵ For such reactions, nickel uses a variety of oxidants including oxygen,^{134, 141, 144} superoxide,¹⁴⁶ hydrogen peroxide,^{135–138, 140, 146–149} and organic hydroperoxides.^{139, 144, 150} Of great importance when considering the chemistry of nickel, the metal-associated ligands play critical roles. Cellular nickel does not exist in a fully aquated state, and the variety of adventitious ligands which bind nickel can have a profound impact on the ability of the metal to produce reactive oxygen species. Indeed, nickel bound to cysteine,^{144, 148} histidine,¹⁴⁸ glutathione,^{139, 148, 150} small peptides,^{146, 147, 150} and amine-containing compounds^{144, 149} accelerated the rates at which nickel creates oxidative damage. On the other hand, the ability of a ligand to act as a prooxidant depends on a low molar ratio to nickel which is more likely to provide an open coordination site for the binding of the oxidant.¹⁴⁸

3.3.2 *In vivo* studies—While there is a dearth of microbial studies pertaining to the role of nickel in cellular oxidative stress, some evidence suggests that such stress may occur during nickel overload. In proteomics studies that investigated the effect of nickel toxicity on *Burkholderia vietnamiensis*¹⁵¹ and *P. putida*⁸⁸ the number of oxidative stress-associated defense proteins was found to increase. For example, *B. vietnamiensis* cells stressed with 3.4 mM Ni(II) exhibited increases in the level of superoxide dismutase (SOD), a superoxide scavenger.¹⁵¹ Similarly, nickel-toxified *P. putida* produced increases in SOD as well as thioredoxin, ferredoxin NADP reductase, a thiol-specific antioxidant, GTP-binding protein TypA, and a 2-oxo-acid dehydrogenase.⁸⁸ Furthermore, elimination of cytoplasmic SOD sensitized *E. coli* cells to this metal.¹⁵² Nickel (100 μM) completely inhibited the growth of a *sodA sodB* mutant on complex medium while single mutants were as resistant to nickel as wild-type cells.¹⁵² These data suggest that superoxide may be produced in nickel-toxified cells. In addition to increased SOD levels, nickel-overload led to an increase in two hydrogen peroxide scavengers, catalase¹⁵³ and peroxidase.^{153, 154}

One of the hallmarks of oxidative stress is damage to DNA. Nickel-dependent DNA damage has been documented in higher eukaryotes,⁴⁻⁷ but the evidence in microorganisms is murky. Nickel was not found to cause reverse mutations in yeast, but it did lead to weak conversions.¹⁵⁵ In *S. enterica*, high intracellular nickel did not result in base pair or frameshift mutations,¹⁵⁶ and the metal was found to be equally toxic to wild-type *E. coli* cells and *recA* mutants.¹⁵⁷ In contrast, nickel generated an increase in the mutation rate of *Corynebacterium*.¹⁵⁸ Studies in mammals are beginning to demonstrate that the role of nickel in carcinogenesis may be due to inhibition of DNA repair and not to an acceleration of damage.^{85, 86, 159} Similarly, nickel serves as a comutagen with alkylating agents in microorganisms,¹⁶⁰ likely due to inhibition of DNA repair.^{160, 161} Indeed, nickel inhibits *E. coli* MutT, but with an IC₅₀ of 1.5 mM.¹⁶² Nickel also was shown to inhibit various DNA polymerases *in vitro* with IC₅₀'s ranging from 10 to 780 μ M.¹¹⁴ These data suggest that nickel does not cause a significant amount of oxidative DNA damage *in vivo*, and that any mutagenic effect of nickel is likely due to inhibition of DNA repair.

Oxidation of proteins has not been reported to be a major mechanism of nickel toxicity in microorganisms. Nevertheless, nickel increases the GSSG/GSH ratio¹⁵⁴ which hints at the possibility of nickel-dependent modulation of the oxidation status of cysteinyl thiol in proteins. The importance of this effect is unclear, however, since the levels of GSH reductase were not enhanced upon nickel exposure.¹⁵⁴

Oxidative stress has been shown to cause lipid peroxidation.¹⁶³ Nickel-toxified filamentous fungus *Curvularia lunata* had an increase in membrane lipid saturation and an enhanced level of thiobarbituric acid reactive substances (TBARS)¹⁶⁴. TBARS are lipid degradation products used as an indirect measure of lipid peroxidation. Extensive lipid peroxidation only occurs in membranes containing polyunsaturated fatty acids;^{131, 165} therefore, lipid peroxidation is unlikely to occur in most eubacteria. The requirement for polyunsaturated fatty acids to propagate lipid peroxidation could explain the nickel-dependent increase in lipid saturation level of *C. lunata*.

In summary, the overall importance of oxidative stress in nickel toxicity may be overstated for most microorganisms. For example, oxidative stress cannot account for nickel toxicity of cells grown in the absence of oxygen.¹⁶⁶⁻¹⁶⁸

4.0 Perspectives

Our discussion of nickel toxicity began by enumerating four mechanistic hypotheses to account for microbial damage by this metal. We described several examples where substitution of nickel for the normal metal leads to inactivation of a metalloenzyme. Depending on the specific growth conditions, such inhibition could account for some of the cellular toxicity by nickel. For example, nickel substitution into a particular iron oxygenase could hinder growth on the cognate carbon source. Similarly, we provided samples of non-metalloenzymes that are inhibited by nickel binding to the active site. Nickel is known to bind to Cys and/or His catalytic residues of several enzymes, with consequent reductions in activities. In addition we mentioned several enzyme cases where the inhibitory metal appears to bind to a secondary or allosteric site to affect activity. Much of the scarce

literature on the mechanisms of nickel inhibition relates to the interaction of the metal with specific enzymes; however, nickel toxicosis has yet to be linked to the failure of a specific enzyme.

In contrast to the many examples described above where nickel toxicity involves direct inhibition of enzymes, the evidence related to the fourth hypothesis, that nickel directly causes oxidative stress, is much less compelling. Future work will need to determine if SOD protects *E. coli* from nickel in the absence of oxygen. For example, oxygen-independent protection by SOD has been demonstrated for copper where heterologous expression of the human Cu, Zn SOD in *E. coli* protected cells from anaerobic copper overload.¹⁶⁹ Furthermore, MnSOD has been shown to bind metals other than manganese;^{170, 171} thus MnSOD may act as an adventitious nickel scavenger. If nickel toxicity does indeed lead to oxidative stress, then it will be necessary to determine whether this is a direct or indirect effect. A likely mechanism by which nickel could indirectly cause oxidative stress is through the increase of cellular iron. Iron has long been shown to be the primary metal involved with oxidative stress. The increased demand for iron import during nickel overload⁹² coupled to nickel's ability to damage iron proteins (section 3.1.1) suggest that nickel-toxicity leads to an increase in the intracellular free iron pool. This indirect enhancement of oxidative stress was also hypothesized for copper-generated oxidative stress *in vivo*.¹⁶⁹

In summary, the general importance of nickel as a universal microbial toxicant is being realized due to the discovery of a widely distributed nickel defense system (RcnA) and the prevalence of nickel in industrial pollution. Despite the numerous demonstrations of nickel toxicosis in all forms of life, the mechanism of toxicity in microorganisms is generally unknown and the target will likely vary with the specific physiology of the cell of interest.

Acknowledgments

Studies related to this topic in the Hausinger laboratory are supported by NIH grant DK045686.

References

1. Nriagu, JO. Nickel in the Environment. John Wiley & Sons; New York, NY: 1980.
2. Ansari MI, Malik A. Environ Monit Assess. 2010; 167:151–163. [PubMed: 19548097]
3. Nieminen, TM.; Ukonmaanaho, L.; Rausch, N.; Shoty, W. Metal Ions in Life Sciences. Sigel, A.; Sigel, H.; Sigel, RKO., editors. Vol. 2. John Wiley & Sons, Ltd; New York, NY: 2007. p. 1-30.
4. Denkhau E, Salnikow K. Crit Rev Oncol Hematol. 2002; 42:35–56. [PubMed: 11923067]
5. Kasprzak KS, Sunderman FW Jr, Salnikow K. Mutat Res. 2003; 533:67–97. [PubMed: 14643413]
6. Das KK, Das SN, Dhundasi SA. Indian J Med Res. 2008; 128:412–425. [PubMed: 19106437]
7. Kasprzak, KS.; Salnikow, K. Metal Ions in Life Sciences. Sigel, A.; Sigel, H.; Sigel, RKO., editors. Vol. 2. John Wiley & Sons, Ltd; New York, NY: 2007. p. 619-660.
8. Yusuf M, Fariduddin Q, Hayat S, Ahmad A. Bull Environ Contam Toxicol. 2011; 86:1–17. [PubMed: 21170705]
9. Babich H, Stotzy G. Adv Appl Microbiol. 1983; 29:195–265. [PubMed: 6650263]
10. Rodrigue A, Effantin G, Mandrand-Berthelot MA. J Bacteriol. 2005; 187:2912–2916. [PubMed: 15805538]
11. Barceloux DG. J Toxicol Clin Toxicol. 1999; 37:239–258. [PubMed: 10382559]

12. Mulrooney SB, Hausinger RP. FEMS Microbiol Rev. 2003; 27:239–261. [PubMed: 12829270]
13. Li Y, Zamble DB. Chem Rev. 2009; 109:4617–4643. [PubMed: 19711977]
14. Ragsdale SW. J Biol Chem. 2010; 284:18571–18575. [PubMed: 19363030]
15. Kaluarachchi H, Chung KCC, Zamble DB. Nat Prod Rep. 2010; 27:681–694. [PubMed: 20442959]
16. Guldán H, Sterner R, Babinger P. Biochemistry. 2008; 47:7376–7384. [PubMed: 18558723]
17. Merkens H, Kappl R, Jakob RP, Schmid FX, Fetzner S. Biochemistry. 2008; 47:12185–12196. [PubMed: 18950192]
18. Dosanjh NS, Michel SLJ. Curr Opin Chem Biol. 2006; 10:123–130. [PubMed: 16504569]
19. Iwig JS, Chivers PT. Nat Prod Rep. 2010; 27:658–667. [PubMed: 20442957]
20. Dannielli A, Scarlato V. FEMS Microbiol Rev. 2010; 34:738–752. [PubMed: 20579104]
21. Reyes-Caballero H, Campanello GC, Giedroc DP. Biophys Chem. 2011; 156:103–114. [PubMed: 21511390]
22. Blériot C, Effantin G, Lagarde F, Mandrand-Berthelot MA, Rodrique A. J Bacteriol. 2011; 193:3785–3793. [PubMed: 21665978]
23. Finney LA, O'Halloran TV. Science. 2003; 300:931–936. [PubMed: 12738850]
24. Outten CE, O'Halloran TV. Science. 2001; 292:2488–2492. [PubMed: 11397910]
25. De Pina K, Desjardin V, Mandrand-Berthelot MA, Giordano G, Wu LF. J Bacteriol. 1999; 181:670–674. [PubMed: 9882686]
26. Chivers PT, Sauer RT. Protein Sci. 1999; 8:2494–2500. [PubMed: 10595554]
27. Schreiter ER, Sintchak MD, Guo Y, Chivers PT, Sauer RT, Drennan CL. Nature Struct Biol. 2003; 10:794–799. [PubMed: 12970756]
28. Wang SC, Dias AV, Zamble DB. Dalton Trans. 2009
29. Chivers PT, Sauer RT. J Biol Chem. 2000; 275:19735–19741. [PubMed: 10787413]
30. West AL, StJohn F, Lopes PEM, MacKerell AD Jr, Pozharski E, Michel SLJ. J Am Chem Soc. 2010; 132:14447–14456. [PubMed: 20863122]
31. Benanti EL, Chivers PT. J Biol Chem. 2007; 282:20365–20375. [PubMed: 17522054]
32. Dosanjh NS, Hammerbacher NA, Michel SL. Biochemistry. 2007; 46:2520–2529. [PubMed: 17291009]
33. Muller C, Bahlawane C, Aubert S, Delay CM, Schauer K, Michaud-Soret I, De Reuse H. Nucleic Acids Research. 2011 in press.
34. Iwig JS, Rowe JL, Chivers PT. Molec Microbiol. 2006; 62:252–262. [PubMed: 16956381]
35. Campbell DR, Chapman KE, Waldron KJ, Tottey S, Kendall S, Cavallaro G, Andreini C, Hinds J, Stoker NG, Robinson NJ, Cavet JS. J Biol Chem. 2007; 282:32298–32310. [PubMed: 17726022]
36. Cavet JS, Meng W, Pennella MA, Appelhoff RJ, Giedroc DP, Robinson NJ. J Biol Chem. 2002; 277:38441–38448. [PubMed: 12163508]
37. Zhang Y, Rodionov DA, Gelfand MS, Gladyshev VN. BMC Genomics. 2009; 10:78. [PubMed: 19208259]
38. Eitinger T, Mandrand-Berthelot MA. Arch Microbiol. 2000; 173:1–9. [PubMed: 10648098]
39. Eitinger T, Suhr J, Moore L, Smith JA. Biometals. 2005; 18:399–405. [PubMed: 16158232]
40. Navarro C, Wu LF, Mandrand-Berthelot MA. Mol Microbiol. 1993; 9:1181–1191. [PubMed: 7934931]
41. Cherrier MV, Cavazza C, Bochot C, Lemaire D, Fontecilla-Camps JC. Biochemistry. 2008; 47:9937–9943. [PubMed: 18759453]
42. Jubier-Maurin V, Rodrigue A, Ouahrani-Bettache S, Layssac M, Mandrand-Berthelot MA, Kohler S, Liautard JP. J Bacteriol. 2001; 183:426–434. [PubMed: 11133934]
43. Park KS, Iida T, Yamaichi Y, Oyagi T, Yamamoto K, Honda T. Infect Immun. 2000; 68:5742–5748. [PubMed: 10992480]
44. Beckwith CS, McGee DJ, Mobley HL, Riley LK. Infect Immun. 2001; 69:5914–5920. [PubMed: 11500473]
45. Sebbane F, Mandrand-Berthelot MA, Simonet M. J Bacteriol. 2002; 184:5706–5713. [PubMed: 12270829]

46. Hiron A, Posterara B, Carrière M, Remy L, Delporte C, La Sorda M, Sanguinetti M, Juillard V, Brorezée-Durant E. *Molec Microbiol.* 2010; 77:1246–1260. [PubMed: 20662775]
47. Wu LF, Mandrand-Berthelot MA, Waugh R, Edmonds CJ, Holt SE, Boxer DH. *Mol Microbiol.* 1989; 3:1709–1718. [PubMed: 2695744]
48. Eberz G, Eitinger T, Friedrich B. *J Bacteriol.* 1989; 171:1340–1345. [PubMed: 2646280]
49. Eitinger T, Friedrich B. *J Biol Chem.* 1991; 266:3222–3227. [PubMed: 1847142]
50. Wolfram L, Friedrich B, Eitinger T. *J Bacteriol.* 1995; 177:1840–1843. [PubMed: 7896709]
51. Eitinger T, Friedrich B. *Mol Microbiol.* 1994; 12:1025–1032. [PubMed: 7934894]
52. Komeda H, Kobayashi M, Shimizu S. *Proc Natl Acad Sci.* 1997; 94:36–41. [PubMed: 8990157]
53. Degen O, Kobayashi M, Shimizu S, Eitinger T. *Arch Microbiol.* 1999; 171:139–145. [PubMed: 10201093]
54. Fu C, Javedan S, Moshiri F, Maier RJ. *Proc Natl Acad Sci.* 1994; 91:5099–5103. [PubMed: 8197192]
55. Mobley HL, Garner RM, Bauerfeind P. *Mol Microbiol.* 1995; 16:97–109. [PubMed: 7651142]
56. Cole ST, Brosch R, Parkhill J, Garnier T, Churcher C, Harris D, Gordon SV, Eiglmeier K, Gas S, Barry CE 3rd, Tekaia F, Badcock K, Basham D, Brown D, Chillingworth T, Connor R, Davies R, Devlin K, Feltwell T, Gentles S, Hamlin N, Holroyd S, Hornsby T, Jagels K, Krogh A, McLean J, Moule S, Murphy L, Oliver K, Osborne J, Quail MA, Rajandream MA, Rogers J, Rutter S, Seeger K, Skelton J, Squares R, Squares S, Sulston JE, Taylor K, Whitehead S, Barrell BG. *Nature.* 1998; 393:537–544. [PubMed: 9634230]
57. Eitinger T, Degen O, Böhnke U, Müller M. *J Biol Chem.* 2000; 275:18029–18033. [PubMed: 10748059]
58. Niegowski D, Eshaghi S. *Cell Mol Life Sci.* 2007; 64:2564–2574. [PubMed: 17619822]
59. Schauer K, Gouget B, Carriere M, Labigne A, de Reuse H. *Mol Microbiol.* 2007; 63:1054–1068. [PubMed: 17238922]
60. Davis GS, Flannery EL, Mobley HL. *Infect Immun.* 2006; 74:6811–6820. [PubMed: 17030579]
61. Rodionov DA, Hebbeln P, Gelfand MS, Eitinger T. *J Bacteriol.* 2006; 188:317–327. [PubMed: 16352848]
62. Schauer K, Rodionov DA, de Reuse H. *Trends Biochem Sci.* 2008; 33:330–338. [PubMed: 18539464]
63. Nies DH. *FEMS Microbiol Rev.* 2003; 27:313–339. [PubMed: 12829273]
64. Schmidt T, Schlegel HG. *FEMS Microbiol Ecol.* 1989; 62:315–328.
65. Diels L, Van Roy S, Taghavi S, Van Houdt R, Antonie Van Leeuwenhoek. 2009; 96:247–258. [PubMed: 19582590]
66. Stahler FN, Odenbreit S, Haas R, Wilrich J, Van Vliet AH, Kusters JG, Kist M, Bereswill S. *Infect Immun.* 2006; 74:3845–3852. [PubMed: 16790756]
67. Liesegang H, Lemke K, Siddiqui RA, Schlegel HG. *J Bacteriol.* 1993; 175:767–778. [PubMed: 8380802]
68. Schmidt T, Schlegel HG. *J Bacteriol.* 1994; 176:7045–7054. [PubMed: 7961470]
69. Haney CJ, Grass G, Frake S, Rensing C. *J Ind Microbiol Biotechnol.* 2005; 32:215–226. [PubMed: 15889311]
70. Grass G, Fan B, Rosen BP, Lemke K, Schlegel HG, Rensing C. *J Bacteriol.* 2001; 183:2803–2807. [PubMed: 11292799]
71. Park JE, Schlegel HG, Rhie HG, Lee HS. *Int Microbiol.* 2004; 7:27–34. [PubMed: 15179604]
72. Munkelt D, Grass G, Nies D. *J Bacteriol.* 2004; 186:8036–8043. [PubMed: 15547276]
73. Tso WW, Adler J. *J Bacteriol.* 1974; 118:560–576. [PubMed: 4597449]
74. Borrok D, Borrok MJ, Fein JB, Kiessling LL. *Environ Sci Technol.* 2005; 39:5227–5233. [PubMed: 16086452]
75. Englert DL, Adase CA, Jayaraman A, Manson MD. *J Bacteriol.* 2010; 192:2633–2637. [PubMed: 20233931]
76. Joho M, Inoue M, Tohoyama H, Murayama T. *FEMS Microbiol Lett.* 1990; 66:333–338. [PubMed: 2182379]

77. Joho M, Ishikawa Y, Kunikane M, Inouhe M, Tohoyama H, Murayama T. *Microbios*. 1992; 71:149–159. [PubMed: 1360616]
78. Pearce DA, Sherman F. *J Bacteriol*. 1999; 181:4774–4779. [PubMed: 10438744]
79. Ruotolo R, Marchini G, Ottonello S. *Genome Biol*. 2008; 9:R67. [PubMed: 18394190]
80. Arita A, Zhou X, Ellen TP, Liu X, Bai J, Rooney JP, Kurtz A, Klein CB, Dai W, Begley TJ, Costa M. *BMC Genomics*. 2009; 10:524. [PubMed: 19917080]
81. Fortin D, Southam G, Beveridge TJ. *FEMS Microbiol Ecol*. 1994; 14:121–132.
82. Tripathi VN, Srivastava S. *Can J Microbiol*. 2006; 52:287–292. [PubMed: 16699578]
83. Sar P, Kazy SK, Singh SP. *Lett Appl Microbiol*. 2001; 32:257–261. [PubMed: 11298937]
84. Zadvornyy OA, Allen M, Brumfield SK, Varpness Z, Boyd ES, Zorin NA, Serebriakova L, Douglas T, Peters JW. *Environ Sci Technol*. 2010; 44:834–840. [PubMed: 19928895]
85. Chen H, Costa M. *Biometals*. 2009; 22:191–196. [PubMed: 19096759]
86. Chen H, Giri NC, Zhang R, Yamane K, Zhang Y, Maroney M, Costa M. *J Biol Chem*. 2010; 285:7374–7383. [PubMed: 20042601]
87. Sekirnik R, Rose NR, Mecnovic J, Schofield CJ. *Metallomics*. 2010; 2:397–399. [PubMed: 21072385]
88. Cheng Z, Wei YY, Sung WW, Glick BR, McConkey BJ. *Proteome Sci*. 2009; 7:18. [PubMed: 19422705]
89. Kuchar J, Hausinger RP. *Chem Rev*. 2004; 104:509–526. [PubMed: 14871133]
90. Koch D, Nies DH, Grass G. *Biometals*. 2007; 20:759–771. [PubMed: 17120142]
91. McHugh JP, Rodriguez-Quinones F, Abdul-Tehrani H, Svistunenko DA, Poole RK, Cooper CE, Andrews SC. *J Biol Chem*. 2003; 278:29478–29486. [PubMed: 12746439]
92. Wang S, Wu Y, Outten FW. *J Bacteriol*. 2010; 193:563–574. [PubMed: 21097627]
93. Hausinger RP. *Crit Rev Biochem Molec Biol*. 2004; 39:21–68. [PubMed: 15121720]
94. Purpero VM, Moran GR. *J Biol Inorg Chem*. 2007; 12:587–601. [PubMed: 17431691]
95. Rose NR, McDonough MA, King ONF, Kawamura A, Schofield CJ. *Chem Soc Rev*. 2011 ??
96. Kalliri E, Grzyska PK, Hausinger RP. *Biochem Biophys Res Commun*. 2005; 338:191–197. [PubMed: 16165092]
97. Bankel L, Lindstedt G, Lindstedt S. *J Biol Chem*. 1972; 247:6128–6134. [PubMed: 4265566]
98. Costa M, Davidson TL, Chen H, Ke Q, Zhang P, Yan Y, Huang C, Kluz T. *Mutat Res*. 2005; 592:79–88. [PubMed: 16009382]
99. Chen YP, Dilworth MJ, Glenn AR. *Arch Microbiol*. 1984; 138:187–190.
100. Chen YP, Glenn AR, Dilworth MJ. *Arch Microbiol*. 1985; 141:225–228.
101. Sheppard DE, Penrod JT, Bobik T, Kofoed E, Roth JR. *J Bacteriol*. 2004; 186:7635–7644. [PubMed: 15516577]
102. Buan NR, Escalante-Semerena JC. *J Biol Chem*. 2006; 281:16971–16977. [PubMed: 16636051]
103. Buan NR, Suh SJ, Escalante-Semerena JC. *J Bacteriol*. 2004; 186:5708–5714. [PubMed: 15317775]
104. Kofoed E, Rappleye C, Stojiljkovic I, Roth J. *J Bacteriol*. 1999; 181:5317–5329. [PubMed: 10464203]
105. Seffernick JL, McTavish H, Osborne JP, de Souza ML, Sadowsky MJ, Wackett LP. *Biochemistry*. 2002; 41:14430–14437. [PubMed: 12450410]
106. Niemirowicz G, Parussini F, Aguero F, Cazzulo JJ. *Biochem J*. 2007; 401:399–410. [PubMed: 17007610]
107. Arima J, Iwabuchi M, Hatanaka T. *Biochem Biophys Res Commun*. 2004; 317:531–538. [PubMed: 15063790]
108. Lowther WT, Matthews BW. *Chem Rev*. 2002; 102:4581–4608. [PubMed: 12475202]
109. Chevrier B, D’Orchymont H, Schalk C, Tarnus C, Moras D. *Eur J Biochem*. 1996; 237:393–398. [PubMed: 8647077]
110. Simitopoulou M, Vafopoulou A, Choli-Papadopoulou T, Alichanidis E. *Appl Environ Microbiol*. 1997; 63:4872–4876. [PubMed: 9406407]

111. Dolence JM, Steward LE, Dolence EK, Wong DH, Poulter CD. *Biochemistry*. 2000; 39:4096–4104. [PubMed: 10747800]
112. Sato K, Okubo A, Yamazaki S. *J Biochem*. 1998; 124:51–54. [PubMed: 9644245]
113. Debussche L, Couder M, Thibaut D, Cameron B, Crouzet J, Blanche F. *J Bacteriol*. 1992; 174:7445–7451. [PubMed: 1429466]
114. Snow ET, Xu LS, Kinney PL. *Chem Biol Interact*. 1993; 88:155–173. [PubMed: 8403077]
115. Nakai T, Hasegawa T, Yamashita E, Yamamoto M, Kumasaka T, Ueki T, Nanba H, Ikenaka Y, Takahashi S, Sato M, Tsukihara T. *Structure*. 2000; 8:729–737. [PubMed: 10903946]
116. Wang WC, Hsu WH, Chien FT, Chen CY. *J Mol Biol*. 2001; 306:251–261. [PubMed: 11237598]
117. Louwrier A, Knowles CJ. *Enzyme Micro Technol*. 1996; 19:562–571.
118. Barrett AJ, Rawlings ND. *Biol Chem*. 2001; 382:727–733. [PubMed: 11517925]
119. Cummins PM, O'Connor B. *Int J Biochem Cell Biol*. 1996; 28:883–893. [PubMed: 8811836]
120. Schaeffer M, de Miranda A, Mottram JC, Coombs GH. *Mol Biochem Parasitol*. 2006; 150:318–329. [PubMed: 17028008]
121. Barkay T, Miller SM, Summers AO. *FEMS Microbiol Rev*. 2003; 27:355–384. [PubMed: 12829275]
122. Ghosh S, Sadhukhan PC, Chaudhuri J, Ghosh DK, Mandal A. *J Appl Microbiol*. 1999; 86:7–12.
123. Hurwitz J, Gold M, Anders M. *J Biol Chem*. 1964; 239:3474–3482. [PubMed: 14245405]
124. Urbonavicius J, Jager G, Bjork GR. *Nucl Acids Res*. 2007; 35:3297–3305. [PubMed: 17459887]
125. Liu Y, Santi DV. *Proc Natl Acad Sci*. 2000; 97:8263–8265. [PubMed: 10899996]
126. Sommaruga S, Gioia LD, Tortora P, Polissi A. *Biochem Biophys Res Commun*. 2009; 388:222–227. [PubMed: 19664604]
127. Meredith TC, Woodard RW. *J Biol Chem*. 2003; 278:32771–32777. [PubMed: 12805358]
128. Bongaerts GP, Uitzetter J, Brouns R, Vogels GD. *Biochim Biophys Acta*. 1978; 527:348–358. [PubMed: 728443]
129. Imhoff RD, Power NP, Borrok MJ, Tipton PA. *Biochemistry*. 2003; 42:4094–4100. [PubMed: 12680763]
130. Gabison L, Chiadmi M, El Hajji M, Castro B, Colloc'h N, Prange T. *Acta Crystallogr D Biol Crystallogr*. 66:714–724. [PubMed: 20516624]
131. Imlay JA. *Annu Rev Microbiol*. 2003; 57:395–418. [PubMed: 14527285]
132. Imlay JA. *Annu Rev Biochem*. 2008; 77:755–776. [PubMed: 18173371]
133. Valko M, Morris H, Cronin MT. *Curr Med Chem*. 2005; 12:1161–1208. [PubMed: 15892631]
134. Cheng CC, Rokita SE, Burrows CJ. *Ang Chem Internat Ed*. 1993; 32:277–278.
135. Kawanishi S, Inoue S, Yamamoto K. *Carcinogenesis*. 1989; 10:2231–2235. [PubMed: 2686851]
136. Lloyd DR, Phillips DH. *Mutat Res*. 1999; 424:23–36. [PubMed: 10064847]
137. Kasprzak KS, Hernandez L. *Cancer Res*. 1989; 49:5964–5968. [PubMed: 2790811]
138. Kaczmarek P, Szczepanik W, Jezowska-Bojczuk M. *Dalton Trans*. 2005:3653–3657. [PubMed: 16258616]
139. Shi X, Mao Y, Ahmed N, Jiang H. *J Inorg Biochem*. 1995; 57:91–102. [PubMed: 7861128]
140. Kelly MC, Whitaker G, White B, Smyth MR. *Free Radic Biol Med*. 2007; 42:1680–1689. [PubMed: 17462536]
141. Butzow JJ, Eichhorn GL. *Biopolymers*. 1965; 3:95–107. [PubMed: 5889538]
142. Bal W, Liang R, Lukszo J, Lee SH, Dizdaroqlu M, Kasprzak KS. *Chem Res Toxicol*. 2000; 13:616–624. [PubMed: 10898594]
143. Gill G, Richter-Rusli AA, Ghosh M, Burrows CJ, Rokita SE. *Chem Res Toxicol*. 1997; 10:302–309. [PubMed: 9084910]
144. Shi X, Dalal NS, Kasprzak KS. *J Inorg Biochem*. 1993; 15:211–225. [PubMed: 8388916]
145. Repetto MG, Ferrarotti NF, Boveris A. *Arch Toxicol*. 2010; 84:255–262. [PubMed: 19936709]
146. Cotellet N, Trémolières E, Bernier JL, Catteau JP, Hénichart JP. *J Inorg Biochem*. 1992; 46:7–15. [PubMed: 1315842]
147. Torreilles J, Guérin MC. *FEBS Letters*. 1990; 272:58–60. [PubMed: 2172019]

148. Joshi S, Husain MM, Chandra R, Hasan SK, Srivastava RC. Hum Exp Toxicol. 2005; 24:13–17. [PubMed: 15727051]
149. Sigel H. J Coord Chem. 1974; 3:235–247.
150. Shi X, Dalal NS, Kasprzak KS. Arch Biochem Biophys. 1992; 299:154–162. [PubMed: 1332613]
151. Van Nostrand JD, Arthur JM, Kilpatrick LE, Neely BA, Bertsch PM, Morris PJ. Microbiology. 2008; 154:3813–3824. [PubMed: 19047749]
152. Geslin C, Llanos J, Prieur D, Jeanthou C. Res Microbiol. 2001; 152:901–905. [PubMed: 11766965]
153. Healy WB, Cheng SC, Mc EW. Arch Biochem Biophys. 1955; 54:206–214. [PubMed: 13229372]
154. Randhawa VK, Zhou F, Jin X, Nalewajko C, Kushner DJ. Can J Microbiol. 2001; 47:987–993. [PubMed: 11766059]
155. Singh I. Mutat Res. 1984; 137:47–49. [PubMed: 6379438]
156. Biggart NW, Costa M. Mutat Res. 1986; 175:209–215. [PubMed: 3537781]
157. Brandi G, Schiavano GF, Albano A, Cattabeni F, Cantoni O. Mutat Res. 1990; 245:201–204. [PubMed: 2233841]
158. Pikálek P, Necásek J. Folia Microbiol. 1983; 28:17–21. [PubMed: 6832655]
159. Hartwig A, Beyersmann D. Mutat Res. 1989; 217:65–73. [PubMed: 2911267]
160. Dubin JS, LaVelle JM. Mutat Res. 1986; 162:187–199. [PubMed: 3528829]
161. Wozniak K, Blasiak J. Cell Mol Biol Lett. 2004; 9:83–94. [PubMed: 15048153]
162. Porter DW, Yakushiji H, Nakabeppu Y, Sekiguchi M, Fivash MJ Jr, Kasprzak KS. Carcinogenesis. 1997; 18:1785–1791. [PubMed: 9328176]
163. Niki E, Yoshida Y, Saito Y, Noguchi N. Biochem Biophys Res Commun. 2005; 338:668–676. [PubMed: 16126168]
164. Paraszewicz K, Bernat P, Naliwajski M, Dlugonski J. Arch Microbiol. 2010; 192:135–141. [PubMed: 20047047]
165. Bielski BH, Arudi RL, Sutherland MW. J Biol Chem. 1983; 258:4759–4761. [PubMed: 6833274]
166. Wu LF, Navarro C, de Pina K, Quénard M, Mandrand MA. Environ Health Perspect. 1994; 102(Suppl 3):297–300. [PubMed: 7843119]
167. Zayed G, Winter J. Appl Environ Microbiol. 2000; 53:726–731.
168. Bartacek J, Ferosa FG, Catena AB, Lens PN. J Hazard Mater. 2010; 180:289–296. [PubMed: 20471748]
169. Macomber L, Imlay JA. Proc Natl Acad Sci. 2009; 106:8344–8349. [PubMed: 19416816]
170. Beyer WF Jr, Fridovich I. J Biol Chem. 1991; 266:303–308. [PubMed: 1985901]
171. Whittaker MM, Whittaker JW. Biochemistry. 1997; 36:8923–8931. [PubMed: 9220980]
172. O'Brien JR, Schuller DJ, Yang VS, Dillard BD, Lanzilotta WN. Biochemistry. 2003; 42:5547–5554. [PubMed: 12741810]

Fig. 1.
A representation of selected nickel trafficking pathways in bacteria.

Fig. 2.

A schematic representation of potential mechanisms of nickel toxicity. Mechanisms are divided into three categories: direct mechanisms with multiple lines of evidence (black lines), direct mechanisms with few lines of evidence (dashed lines), and indirect mechanisms (blue lines).

Fig. 3. Nickel damage involving substitution of the functional metal in a metalloenzyme. The structure is shown in cartoon mode for one subunit of the iron(II)- and α -ketoglutarate-dependent *E. coli* protein TauD (PDB access code 1os7).¹⁷² Nickel (green sphere) is known to inhibit this protein by a slow-binding process involving competition with iron (brown sphere) for its amino acid ligands (shown as sticks with orange carbons) and α -ketoglutarate (sticks with yellow carbons). Figure prepared with PYMOL.

Fig. 4. Nickel damage by binding to a non-metal enzyme. The structure in cartoon mode is shown for *N*-carbamoyl D-amino acid amidohydrolase from *Agrobacterium radiobacter* (PDB access code 1fo6)¹¹⁶. Nickel (green sphere) is likely to inhibit the protein by binding to some combination of three active site residues (depicted as sticks with orange carbon atoms) and three nearby histidine residues (sticks with red carbons). Figure prepared with PYMOL.

Table 1

Representative examples of microbial enzymes inhibited by nickel

Active Site	Enzyme	Organism	[Ni(II)] μ M	Inactivation (%)	Ref
Iron	taurine/ α KG dioxygenase	<i>E. coli</i>	32	50	96
	thymidine 2' hydroxylase	<i>N. crassa</i>	250	95%	97
	protocatechuate 3,4-dioxygenase	<i>R. leguminosarum</i>	10	85	99
	catechol 1,2-dioxygenase	<i>R. leguminosarum</i>	100	50	100
	ATP-cob(I)alamin adenosyltransferase	<i>S. enterica</i>	100	50	102
	atrazine chlorohydrolase	<i>Pseudomonas sp.</i>	200	60	105
	metallocarboxypeptidase (MCP-1)	<i>T. cruzi</i>	10	54	106
	aminopeptidase	<i>S. septatus</i>	100	47	107
Zinc	aminopeptidase	<i>S. griseus</i>	100	60	107
	tripeptidase	<i>P. pentosaceus</i>	100	94	110
	prenyl protease (Rce 1p)	<i>S. cerevisiae</i>	87	48	111
	nitrous oxide reductase	<i>R. sphaeroides</i>	100	50	112
Copper	cobaltochelatase	<i>P. denitrificans</i>	3	50	113
Magnesium	DNA polymerase I (Klenow fragment)	<i>E. coli</i>	370	50	114
	DNA polymerase	T4 bacteriophage	240	50	114
	DNA polymerase	T7 bacteriophage	780	50	114
	<i>N</i> -carbamoyl <i>D</i> -amino acid amidohydrolase	<i>Agrobacterium radiobacter</i>	0.1	NS ^b	117
Non-metal	pyroglutamyl peptidase I	<i>L. major</i>	100	48	120
	mercury(II) reductase	<i>A. chroococcum</i>	100	62	122
	cytosine-5 methyltransferase	<i>E. coli</i>	1	80	123
	uracil-5 methyltransferase	<i>E. coli</i>	1	80	123

^a Cobalt is a substrate for this enzyme, not a required cofactor.

^b NS, 0.1 μ M was said to be inhibitory, but the extent of inhibition was not stated.